THE PHILIPPINE JOURNAL OF SCIENCE

VOL. 55 DECEMBER, 1934 No. 4

HABITATS OF PHILIPPINE ANOPHELES LARVÆ[1]

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FIVE PLATES

INTRODUCTION

The first reference relating to preferential breeding places of Philippine *Anopheles* appears to be that of Crosby(1) and Whitmore(2) as reported by Russell.(3) Other reports have come from Ludlow,(4) Banks,(5) Barber et al.,(6) Tiedemann,(7) Mieldazis,(8) Manalang,(9) Ejercito,(10) Baisas,(11) Holt and Russell,(12) King,(13) and Russell.(14)

These papers, of course, have been considered in preparing the article which we are presenting, but our report is based essentially on the collections of the staff of Malaria Investigations during the period from January, 1930, to September, 1934.(12, 14) These collections have been made in every province in the Philippine Islands, at various seasons, in all types of breeding places and at various altitudes. They form an excellent basis for conclusions regarding the selective breeding habits of local

[1] This paper is based on collections of Malaria Investigations, a project jointly supported by the Bureau of Science, Manila, and the International Health Division of the Rockefeller Foundation. We have had the assistance of Messrs. A. M. Nono and D. Santiago, of Malaria Investigations, in making many of our collections. The United States Army Medical Department Research Board collaborated in a number of field trips.

anopheline larvæ. This paper is presented in conjunction with our practical key to the *Anopheles* larvæ of the Islands.(15)

GENERAL DISCUSSION OF ANOPHELES BREEDING PLACES

It is difficult to classify the breeding places of anopheline mosquitoes, because although many species show preferential breeding habits yet different species grow in association and the same species vary widely. Furthermore, there is at present little information available to indicate why local species are found in their usual habitats or why in unusual places.

The following list presents the chief types of breeding places in the Philippines.

A. Running water.—Creek, stream, and river margins. Flowing ditches, canals, and springs. These breeding places may be shaded or open, clear or muddy, and the water may be moving very slowly or at moderate velocity.

B. Stagnant fresh water.—Lakes, ponds, and grassy pools. Stagnant ditches and waste water. Fresh-water swamps and backwaters. Irrigation or artesian-well water from overflows or leaks. Residual river pools. Wells, cisterns, and artificial ponds.

C. Small collections of fresh water.—Tree holes, cut stumps, and collections among roots of trees. Pools in rocks. Temporary rain puddles, hoof and wheel tracks. Miscellaneous barrels, tubs, etc.

D. Rice fields.—The fields may be in cultivation or fallow. The water may be stagnant or have a perceptible current.

E. Salt water.—Salt beds, fishponds, and lagoons. Brackish swamps and river mouths.

BREEDING PLACES OF PHILIPPINE ANOPHELES

The breeding places of Philippine *Anopheles* will be considered species by species.

1. Anopheles aitkeni var. bengalensis.—Collected in Luzon, Mindanao, and Palawan. Breeds mainly in clear, cool, forest streams, along shaded edges where there is little or no current. It is not a common species, and even where found it is generally taken in small numbers.

2. Anopheles baezai (variety?).—Collected in Mindanao, Cagayan de Sulu, Capiz, Camarines Norte, and Palawan. Breeds only in pools of brackish water, shaded or exposed, with or without vegetation. This species is neither common nor abundant. It is sometimes associated with *A. litoralis* and *A. subpictus*

var. *indefinitus*. (We are not certain that this is true *baezai*, although the larvæ are identical with published descriptions. It is not typical *umbrosus* as previously reported in Philippine collections.)

3. Anopheles barbirostris.—Collected throughout the entire Archipelago, breeding abundantly in a wide variety of habitats, but not in salt water. It is found among aquatic vegetation and along the quiet edges of streams and rivers, both in the lowlands and mountains, in both shaded and exposed locations. It is abundant in large vegetated ponds and pools formed by springs or by dams, and it is equally prevalent in canals and irrigation ditches.

4. Anopheles filipinæ.—Collected in Luzon from Ilocos to Bicol regions. Also in Mindoro, Mindanao, and Masbate, but not reported from other islands. Neither very abundant nor very common. Most frequently breeds among aquatic plants in spring water, either slowly flowing or impounded. Sometimes taken in small numbers with *A. minimus* var. *flavirostris* along the banks of small streams, canals, or flowing ditches.

5. Anopheles annularis.—Widespread in Luzon, also reported from Misamis. Breeds among aquatic vegetation in large ponds of fresh water, also in slowly flowing ditches and in rice fields. Found also along shallow vegetated edges of lakes. Often associated with *A. philippinensis*, and sometimes with other species.

6. Anopheles gigas var. formosus.—Found in Mountain Province, Luzon, at elevations up to about 7,500 feet, breeding in heavily shaded streams, along the edges or among débris and aquatic plants. Found less abundantly in open grassy streams or canals. Often taken with *A. lindesayi* var. *benguetensis*.

7. Anopheles hyrcanus var. nigerrimus.—Collected from Luzon to Mindanao in rice fields, stagnant vegetated canals, and in impounded water. Not usually associated with *sinensis* but, on the contrary, usually taken where *sinensis* is scarce.

8. Anopheles hyrcanus var. sinensis.—Collected chiefly from Luzon, usually among aquatic vegetation in impounded spring water. Also in slowly flowing vegetated canals and ponds, and along the shallow edges of lakes, not infrequently associated with algæ and *Chara*. One of the few lowland species also found in the mountains, for example, at Baguio (5,000 feet).

9. Anopheles insulæflorum.—Found from Luzon to Mindanao, usually in quiet, shaded, forest streams, often among débris in small nooks where the water is not flowing. Sometimes found in the same stream as *A. minimus* var. *flavirostris* but usually

not exactly in the same location. *Anopheles insulæflorum* seems to prefer parts of the stream bank where the water is more nearly stagnant.

10. Anopheles karwari.—Collected from Tayabas, Bulacan, and Laguna. Also from Bukidnon. A rare species in the Philippines. Found in spring pools and occasionally in clear shaded streams with *A. maculatus.*

11. Anopheles kochi.—Found from Luzon to Mindanao but not very abundantly. Breeds usually in small, muddy, open pools, during the rainy season. Also found in unplanted rice fields. Usually associated with *A. vagus* var. *limosus.*

12. Anopheles kolambuganensis.—Found only in a few localities in Mindanao, never abundantly. Breeds in streams within virgin forest, disappearing when the trees are cut and the streams cleared. Usually prefers quiet shaded portions of a stream where there is no direct current. Larvæ are often plainly seen by the collector with unaided eyes, because the white bands on the larvæ stand out in contrast to the dark background of water or débris.

13. Anopheles leucosphyrus.—Found in Luzon and Mindanao but not commonly or abundantly; in fact, this is a rare local species. Breeds in rock holes and stagnant pools in the beds of mountain creeks, in heavily shaded places.

14. Anopheles lindesayi var. benguetensis.—Reported from Luzon, in mountain streams of Baguio and Nueva Ecija and Laguna Provinces. Breeds in well-shaded streams among débris where it is usually easily seen because of the white markings of its body.

15. Anopheles litoralis.—A common and abundant species along the coast of the entire Archipelago. Breeds only in salt or brackish water, in fishponds, salt beds, marshes, and stagnant pools, especially in the midst of algæ.

16. Anopheles ludlowi.—Found from Luzon to Mindanao in fresh-water rivers and streams. Breeds along the edges of streams, open or shaded, especially where the streams widen and stagnate. Most abundant from December to February. May be associated with *A. subpictus* var. *indefinitus* and *A. maculatus.*

17. Anopheles maculatus.—This species is sometimes a carrier of malaria. Found from Luzon to Mindanao, from lowlands to mountains, even up to 5,000 feet, but not abundantly. Most frequently found among algæ at the edges of shaded forest streams. Sometimes associated with *A. ludlowi* and *A. subpictus* var. *indefinitus.*

18 *Anopheles mangyanus.*—Found from Luzon to Mindanao. Breeds in shallow flowing streams with sandy or rocky beds. Prefers clear water, either shaded or exposed. Grows among roots or grasses at the edges of streams, and sometimes in vegetated irrigation ditches. Not found above 2,000 feet. Frequently associated with *A. minimus* var. *flavirostris.*

19. *Anopheles minimus var. flavirostris.*—This species, the chief malaria vector in the Islands, is both common and abundant. We have collected this species in every province in the Philippines excepting Manila, Capiz, Iloilo, Romblon, and Surigao. However, it certainly occurs in every province but Manila. This species breeds particularly in foothill streams along the shaded edges, especially among bamboo roots. It is also sometimes found at the edges of rivers, canals, and irrigation ditches. It has been found in wells and is occasionally taken from stagnant pools where presumably it has been carried by an overflow from its natural breeding place. We have never found it in salt water or in rice fields. We have noticed no essential differences in the breeding habits of this anopheline from Ilocos Norte to Tawitawi and from Samar to Balabac. We have not found it above 2,000 feet altitude.

20. *Anopheles parangensis.*—Found only in Mindanao, as a very rare species. Usually in fresh-water pools formed in the deeper parts or side pools of a drying stream, usually well shaded with abundant vegetation. Occasionally associated with *A. subpictus* var. *indefinitus* and *A. pseudobarbirostris.* In recent collecting trips to northern Mindanao and to Cotabato made specially to obtain *parangensis,* we failed to find a single specimen. In 1933 our collection was extremely meager.

21. *Anopheles philippinensis.*—Found from Luzon to Mindanao, breeding usually in slews (backwaters), large ponds, and impounded water with *Chara, Pistia,* and other aquatic vegetation. Also found in rice fields and stagnated canals and ditches where there is abundant vegetation, and along the vegetated edges of lakes. Often associated with *A. annularis, A. subpictus* var. *indefinitus, A. hyrcanus* var. *sinensis,* and *A. pseudobarbirostris.*

22. *Anopheles pseudobarbirostris.*—Found from Luzon to Mindanao. Usually breeds in impounded water and large, well-vegetated ponds. Also found in ditches, canals, rice fields, and lakes, often associated with *A. barbirostris, A. hyrcanus* var. *sinensis,* and *A. philippinensis.*

23. Anopheles subpictus var. indefinitus.—Found from Luzon to Mindanao. This is the only Philippine species that breeds abundantly in both fresh and salt water. It is collected in slews, impounded water, stagnant parts of rivers, especially where *Chara*, algæ, and *Pistia* abound. Breeds abundantly in salt beds and fishponds, especially during and soon after the rainy season when the salinity of these places is not high.

24. Anopheles tessellatus.—Found in Luzon, Palawan, and Mindanao, neither abundantly nor commonly. Breeds in rice-field pools, also along the banks of streams among aquatic plants, and in small, heavily vegetated pools of spring water. Sometimes associated with *A. maculatus*, *A. hyrcanus* var. *sinensis*, and other stream-breeding species. Found both in lowlands and mountains.

25. Anopheles vagus var. limosus.—Found abundantly and commonly from Luzon to Mindanao, breeding mostly during and soon after the rainy season in small, open, muddy pools, newly plowed rice fields, and muddy, slow-flowing ditches. Often associated with *A. kochi*.

26. Balabac species or variety (?).—Found in Balabac and Iwahig, Palawan. Breeds in fresh-water pools, sometimes in the bed of a drying rocky stream. Water well shaded by forest trees, and containing decaying leaves and débris. Associated with *A. barbirostris* and *A. kochi*.

27. Near-leucosphyrus species or variety (?).—Found only in Mindanao. Breeds in rock holes in the beds of streams. These rock holes vary in diameter from a few inches to several feet. Green algæ are sometimes present where these larvæ are found. Holes well shaded; larvæ scarce and hard to collect. This species is found chiefly in the rainy season. True *leucosphyrus* may be found along the same stream but has not been taken with this "near-*leucosphyrus*" from the same holes.

SUMMARY

This paper summarizes what is known about the habitats of Philippine *Anopheles* larvæ. It is based on collections made by the staff of Malaria Investigations from January, 1930, to September, 1934, in every province in the Philippines under varying conditions as to altitude, type of breeding place, and time of year. In preparing this paper due attention has been paid to the published reports of others. It must be emphasized that the Philippine fauna is very rich in *Anopheles* mosquitoes and that relatively little is known about these important insects.

REFERENCES

1. CROSBY, W. D. A report in Post Camp Stotsenburg medical history (unpublished) (1904) 103–104, 129–131.
2. WHITMORE, E. R. A report in Post Camp Stotsenburg medical history (unpublished) (1904) 135, 141–143.
3. RUSSELL, P. F. Malaria and Culicidæ in the Philippine Islands: history and critical bibliography, 1898–1933. Philip. Dept. Agr. and Commerce Tech. Bull. 1 (1934) 1–109.
4. LUDLOW, C. S. Classification, distribution and seasonal flight of the mosquitoes of the Philippine Islands. Journ. Assoc. Milit. Surg. U. S. 12 (1903) 84–107.

   LUDLOW, C. S. The mosquitoes of the Philippine Islands. Geo. Washington Univ. Wash. D. C. (1908) 1–65. Numerous other references to Ludlow's work given by Russell.(3)
5. BANKS, C. S. Biology of Philippine Culicidae. Philip. Journ. Sci. § A 3 (1908) 235–258. Numerous other references to Banks's work given by Russell.(3)
6. BARBER, M. A., ALFONSO RAQUEL, ARISTON GUZMAN, and A. P. ROSA. Malaria in the Philippine Islands. II. The distribution of the commoner anophelines and the distribution of malaria. Philip. Journ. Sci. § B 10 (1915) 177–245.
7. TIEDEMANN, W. v. D. Malaria in the Philippines. Journ. Prev. Med. 1 (1927) 205–254.
8. MIELDAZIS, J. J. Preferential breeding conditions of Anopheles in the Philippine Islands. Philip. Journ. Sci. 41 (1930) 59–63.
9. MANALANG, C. Malaria inquiry and control in Mindanao and Sulu. Mon. Bull. Philip. Health Serv. 7 (1927) 281–298. Numerous other references to Manalang's works are given by Russell.(3)
10. EJERCITO, A. Summarized report on malaria surveys and control in Mindanao and Sulu. Journ. Philip. Is. Med. Assoc. 9 (1929) 277–283. See other references in Russell.(3)
11. BAISAS, F. E. The barbirostris-hyrcanus group of the Philippine Anopheles. Philip. Journ. Sci. 44 (1931) 425–448. Other references in Russell.(3)
12. HOLT, R. L., and P. F. RUSSELL. Malaria and Anopheles reconnaissance in the Philippines. Philip. Journ. Sci. 49 (1932) 305–371.
13. KING, W. V. Recent observations on malaria and Anopheles in the Philippines. South. Med. Journ. 23 (1930) 445–447. Other references in Russell.(3)
14. RUSSELL, P. F. Malaria and Anopheles reconnaissance in the Philippines, II. Philip. Journ. Sci. 54 (1934) 43–59.
15. RUSSELL, P. F., and F. E. BAISAS. A practical illustrated key to larvæ of Philippine Anopheles. Philip. Journ. Sci. 55 (1934) 307–336.

ILLUSTRATIONS

PLATE 1

FIG. 1. Breeding place of *Anopheles gigas* var. *formosus* and *A. lindesayi* var. *benguetensis*. A stream southeast of Baguio Country Club, Mountain Province, Luzon. Elevation about 4,500 feet.

2. Breeding place of *Anopheles aitkeni* var. *bengalensis*, *A. barbirostris*, *A. maculatus*, and *A. mangyanus* (but not *A. minimus* var. *flavirostris*). Camp Labi, Nueva Ecija Province, Luzon. Elevation about 1,000 feet.

3. Breeding place of *Anopheles barbirostris*, *A. leucosphyrus*, and *A. minimus* var. *flavirostris*. Stream at Agricultural College, Laguna Province, Luzon. Elevation about 500 feet.

PLATE 2

FIG. 1. Breeding place of *Anopheles litoralis*. A salt-water lagoon on Langil Island, Zamboanga.

2. Breeding place of *Anopheles hyrcanus* var. *sinensis*, *A. vagus* var. *limosus*, and *A. kochi*. Rice field near Calauan, Laguna Province, Luzon.

3. Breeding place of *Anopheles tessellatus*, *A. barbirostris*, *A. pseudobarbirostris*, *A. philippinensis*, *A. annularis*, *A. hyrcanus* var. *nigerrimus*, and *A. hyrcanus* var. *sinensis*. A sluggish canal near Calauan, Laguna Province, Luzon.

PLATE 3

FIG. 1. Breeding place of *Anopheles filipinæ*, *A. barbirostris*, *A. pseudobarbirostris*, *A. hyrcanus* var. *sinensis*, and *A. subpictus* var. *indefinitus*. Impounded spring water (tanque), Calauan, Laguna.

2. Breeding place of *Anopheles litoralis* and *A. subpictus* var. *indefinitus*. Salt-water fishpond, Parañaque, Rizal Province, Luzon.

3. Breeding place of *Anopheles ludlowi*, *A. subpictus* var. *indefinitus*, and *A. vagus* var. *limosus*. Marikina River, Marikina, Rizal Province, Luzon.

PLATE 4

FIG. 1. Breeding place of *Anopheles minimus* var. *flavirostris*. Calauan, Laguna Province, Luzon.

2. Breeding place of *Anopheles subpictus* var. *indefinitus* but not of *A. litoralis*. Brackish-water fishpond, Parañaque, Rizal Province, Luzon.

3. Breeding place of *Anopheles baezai*(?). Iwahig Penal Colony, Palawan.

PLATE 5

FIG. 1. Breeding place of *Anopheles kochi* and *A. vagus* var. *limosus*. Rain puddle, Calauan, Laguna Province, Luzon.

2. Breeding place of *Anopheles minimus* var. *flavirostris*. A spring in Iwahig, Palawan. (Foreground with ladder. The stream in the background is also a breeding place of *A. minimus* var. *flavirostris*.)

PLATE 1.

PLATE 2.

PLATE 3.

PLATE 4.

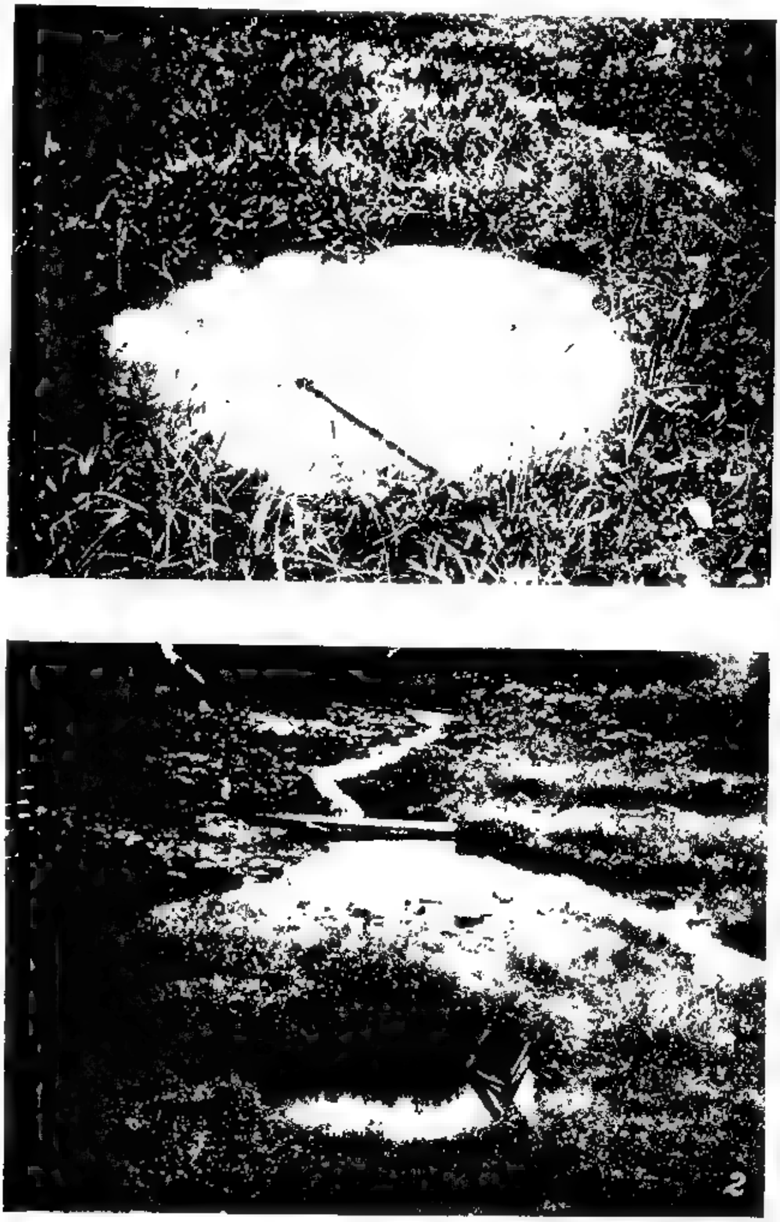

PLATE 5.

A PRACTICAL ILLUSTRATED KEY TO LARVÆ OF PHILIPPINE ANOPHELES [1]

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INTRODUCTION

THIRTY-THREE PLATES AND FIVE TEXT FIGURES

There has long been needed a practical illustrated key to the larvæ of Philippine anopheline mosquitoes. The one presented in this paper has been prepared by the authors during 1934, making use of the collections of the field staff of Malaria Investigations. It is original in that every drawing is a new one, made under the direct supervision of the authors and in that the key has been prepared recently in our laboratory.

Naturally our key is based on the work of many authors who have studied *Anopheles* larvæ common to the Philippines. The work of Ludlow,(1) Banks,(2) Mieldazis,(3) Manalang,(4)

[1] The senior author is chief of Malaria Investigations which is jointly supported by the Bureau of Science, Manila, and the International Health Division of the Rockefeller Foundation. The junior author has been detailed to Malaria Investigations by courtesy of Dr. J. Fajardo, director of the Philippine Bureau of Health. We are indebted to the following for assistance at various times during the year in which we have been preparing this paper: Messrs. Andres M. Nono and Domingo Santiago, Miss Amparo Capistrano, and Mrs. Isabel V. Ramos, all of the staff of Malaria Investigations. All of the drawings are original and were prepared from larvæ caught by the staff of Malaria Investigations. The artists were Miss Lourdes Moskaira (deceased) and Mr. Eliseo Enriquez, to whom we are indebted for painstaking efforts. We would also acknowledge gratefully the assistance of the photographic department of the Bureau of Science.

Baisas, (5) and King (6) has been of importance in developing our knowledge of the Philippine *Anopheles*. The papers by Puri (7) and the recent text by Christophers (8) have been invaluable. The latter gives numerous important references. So too the works of Martini (9) and Root (10) are very useful. Russell (11) gives a complete bibliography of Philippine references.

Our key, of course, is subject to revision and it is not presented as a research study in entomology. In its present form it seems to meet present needs and it has been prepared for practical use.

LARVAL CHARACTERS

Anopheles larvæ possess characteristic hairs which are fairly uniform in fourth-stage specimens. Only larvæ in this last stage, or instar, have been considered in this key. It is not feasible to discuss these characters at length in this paper. Those interested will find good descriptions in Puri (7) and Christophers. (8) We shall merely briefly describe and tabulate below those characters which we have used in our own key.

The hair numbers refer to the numbers assigned to individual hairs by Puri, (7) Martini, (9) and Root. (10) There is a separate series of consecutive numbers for the hairs of each of the following parts of a larva—the head, the prothorax, the mesothorax, the metathorax, and each segment of the abdomen. Dorsal hairs come first and then ventral in the same series (see text figs. 1 to 4).

A. HEAD (TEXT FIG. 1)

1. Clypeal hairs.—These hairs arise on the front of the frons-clypeus. They are as follows:

i. c. Inner clypeal or inner anterior clypeal (hair 2).
o. c. Outer clypeal or outer anterior clypeal (hair 3).
p. c. Posterior clypeal (hair 4).

In the subgenus *Anopheles* the bases of the inner clypeal hairs are closely approximated, often nearly touching. In the subgenus *Myzomyia* these inner clypeals are widely separated, usually twice or more than twice the distance between the bases of the inner and outer hair of the same side.

2. Occipital hairs.—Those we have used are the following:

i. o. Inner occipital or sutural (hair 8).
o. o. Outer occipital or trans-sutural (hair 9).

These hairs are frequently as long as the posterior clypeal. The inner hair is usually simple but may be branched. The outer hair may be simple or feathered.

3. Antennal or shaft hair (hair 11).

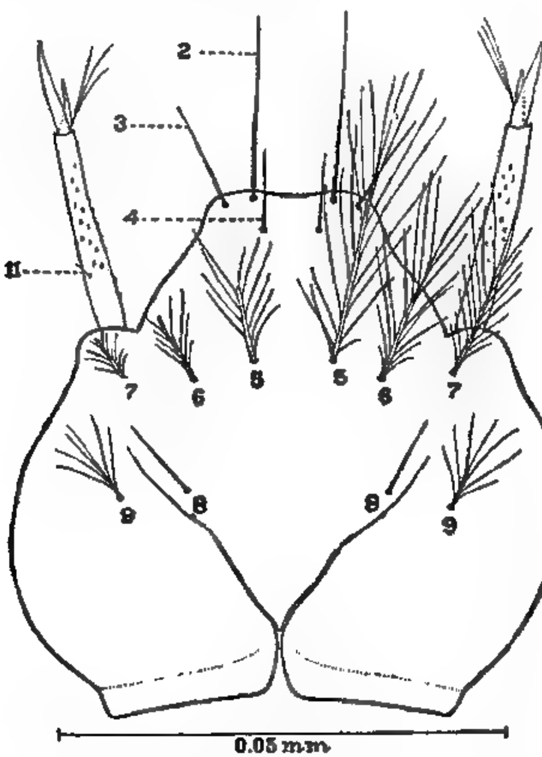

FIG. 1. Head of larva, showing hairs; semidiagrammatic.

B. THORAX (TEXT FIGS. 2 AND 3)

1. Prothoracic hairs.—Those used are the following:

i. s. p. Inner anterior submedian prothoracic or inner submedian prothoracic (hair 11).

m. s. p. Middle anterior submedian prothoracic or middle submedian prothoracic (hair 2).

These, with an outer hair 3, comprise the so-called "shoulder hairs." Usually the middle one is largest and is stout and feathered. The inner hair is sometimes simple but may be branched or feathered.

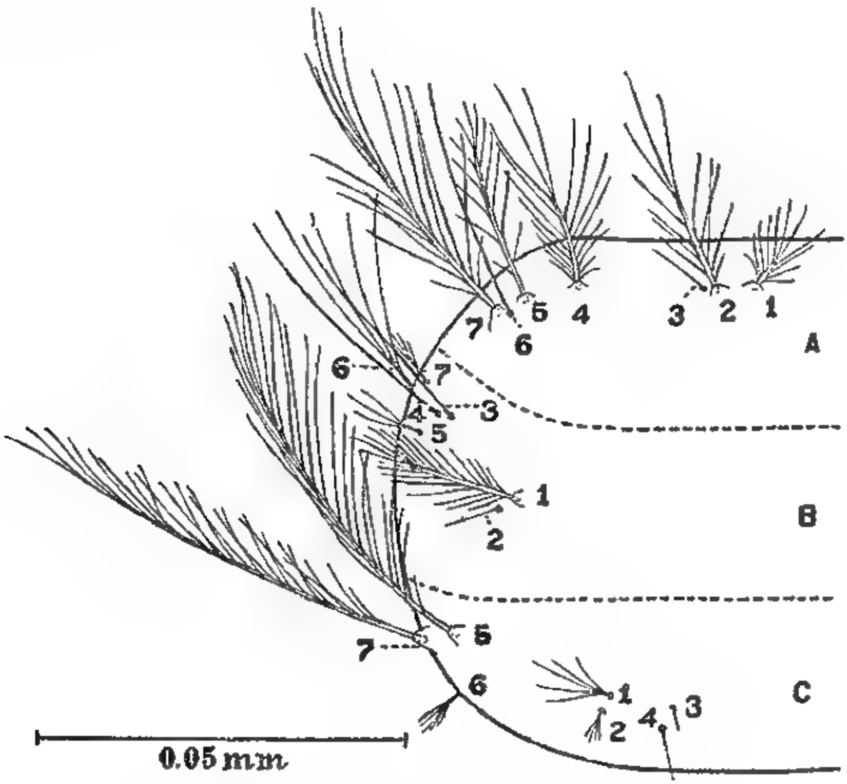

FIG. 2. Dorsum of thorax, showing hairs; semidiagrammatic.

p. v. s. m. Prothoracic ventral submedian or prothoracic (hair 13).
p. t. p. Prothoracic pleural hair group, (hairs 9 to 12).

These pleural hairs are important. They are on the ventrolateral surface of each segment of the thorax and all four in each group arise from a common base. These hairs may be simple or feathered in different combinations. They are useful in separating subgenera.

2. Mesothoracic hairs.—Those used are the following:

m. t. 5. Mesothoracic hair 5. This is one of the small dorsolateral hairs (see text fig. 2).

m. t. p. Mesothoracic pleural hair group (hairs 9 to 12 of Puri and in our text fig. 3, but numbered 10 to 13 by Martini and Root).

3. Metathoracic hairs.—Those used are the following:

m. v. s. m. Metathoracic ventral submedian or metathoracic hair 13.
t. p. Thoracic palmate (hair 1 of Puri and our text fig. 2, but 4 of Martini and Root).

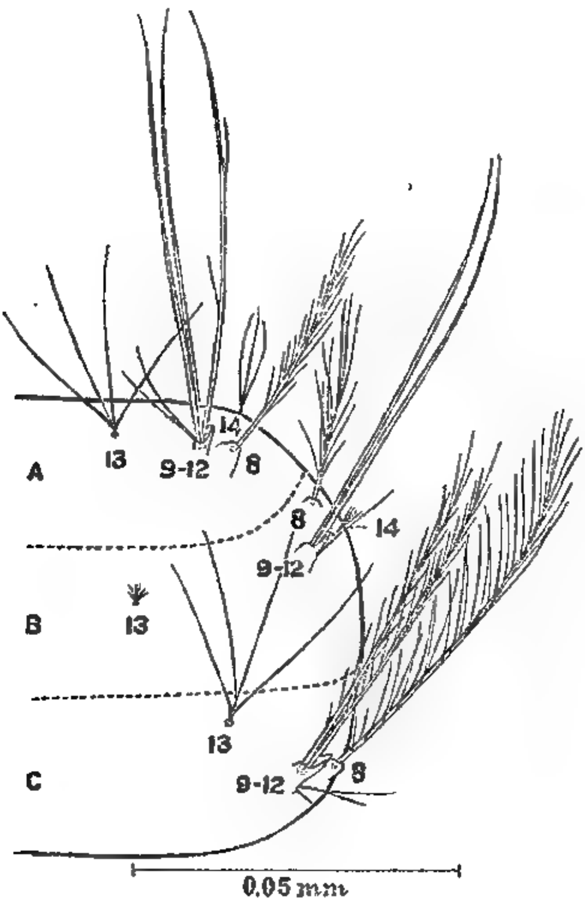

FIG. 3. Venter of thorax, showing hairs; semidiagrammatic.

This thoracic palmate may be simple or have unflattened branches, in which case it is called undeveloped or rudimentary. Frequently it has flattened branches arising together, in which case it is somewhat like an abdominal palmate although never so well developed.

C. ABDOMEN (TEXT FIG. 4)

Abd. pal. Abdominal palmate hairs (or float hairs) on segments I to VII (hair 1).

This hair may be poorly developed, especially on segments I and II. It is developed when it has flattened branches which arise in a whorl from a common base. Well-developed palmate hairs have leaflets which may or may not be serrated or notched and the end portion of which may or may not be in the form of a filament.

a. pal. Antepalmate or prepalmate (hair 2). This hair is posterior to the palmate on segment VI.

d.-l. p. Dorsolateral posterior (hair 5).

lat. Lateral (hairs 6 and 7).

ter. pl. Tergal plates (anterior).

These oval chitinous plates are seen near the anterior border of each abdominal segment. They may be large or small. There are also posterior plates but they are very small. The tergal plates referred to in our own key are the anterior plates. In judging the size of tergal plates refer to those of segments III to VI. The VIIth has a comparatively large plate in all species, and in many species this is also true of the first.

Large: Covers about two-thirds of the width and about one-third of the length of the segment.

Small: Covers at most (usually much less) about one-fifth of the segment either way.

In whole and living specimens an easier way to determine the size of the tergal plate is:

Large: Lateral ends extend far beyond the mid-dark area covered by the alimentary tract and trachial tubes.

Small: Lateral edges barely reach or extend only a little beyond the dark area covered by the alimentary tract and trachial tubes.

pect. Pecten or comb.

The pecten, or comb, is one of the structures around the spiracles. It has long and short projections referred to as teeth.

stig. club. Stigmal club.

We have used this term for the much enlarged chitinous peg lying between the spiracular openings in *pseudobarbirostris*.

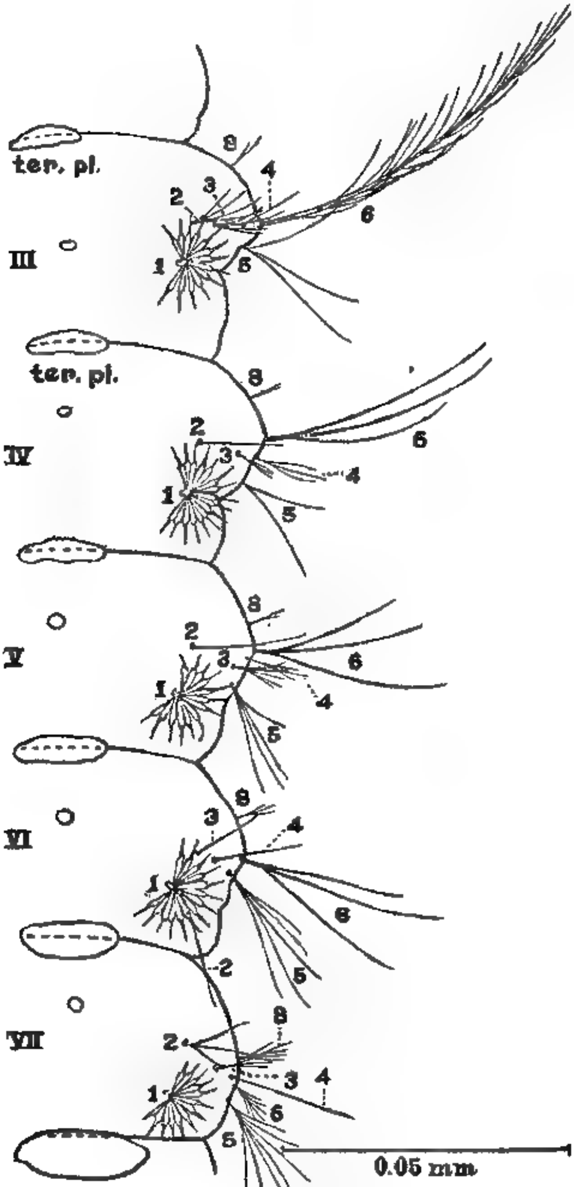

FIG. 4. Dorsal view of abdominal segments III to VII; semidiagrammatic.

It is illustrated in Plate 13 and text fig. 5. For further description see Christophers.(12) What we call stigmal club is referred to by him as "a dense chitinized apex or point anteriorly (of median plate) which lies between the spiracular openings." Imms(13) calls this stigmal club the "chitinous peg" and describes it as "a stout hollow peg of very dark chitin which projects beneath the integument slightly into the cavity of the animal." He notes that the transverse and median plates of the skeleton are attached to this peg. Apparently all *Anopheles*

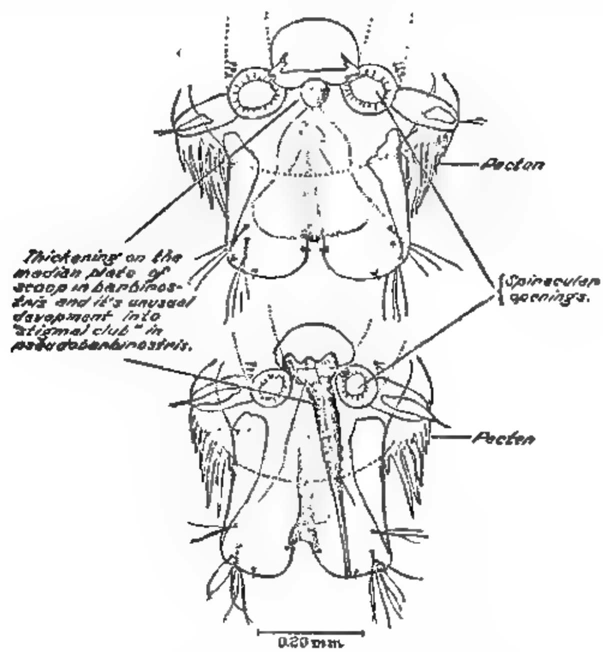

FIG. 5. Spiracular apparatus: *A. barbirostris* and *A. pseudobarbirostris.*

larvæ have this chitinous peg but in *pseudobarbirostris* it is so extraordinarily well developed and prominent that we have referred to it as "stigmal club," present in *pseudobarbirostris* but absent in other species, although as noted above there is a small chitinous peg present in all *Anopheles* larvæ.

In the branching of larval hairs there are, of course, irregularities. This is true of the pilotaxy of *Anopheles* larvæ in other parts of the world. Therefore, one should occasionally expect to see hairs branched which usually are simple and vice versa. Even duplication of hairs is sometimes seen.

In some species the branching of the inner clypeal hairs is so fine that it can be detected only with relatively high magnification. The number of branches is not always the same but we have presented average numbers which should be fair guides. However, sometimes only the stump of a branch remains, the branch itself having been broken off. In such cases considerable care is required to determine the correct branching of the hair in question. The following practical definitions may be of use:

anterior. Towards the head of larva.
posterior. Towards the tail of larva.
ventral. The underside of larva.
dorsal. The top side of larva. This upper side may be recognized by the palmate hairs, or their representatives, on the body and by the occipital hairs on the head.
apical. Towards the outer end of a hair.
basal. Towards the inner end or origin of a hair.
closely approximated and widely separated. The clypeal hairs are closely approximated if their bases are so close that there is scarcely room for another base between them. If more than one base can be put between them they are called widely separated. By another criterion these hairs are closely approximated if the distance between their bases is much less than the distance between the inner and outer clypeal hairs on one side.
dichotomous. Dividing regularly into pairs.
plumose. Resembling a feather.
feathery. With branches coming off the main stem like the branches of a feather, not in pairs.
instar. Stage of development of larva.
serrated. With teethlike irregularities. Notched like a saw.
rachis. Main stem or shaft.
filamented. Having a threadlike prolongation.
confluent. Not distinctly separated but running together almost or quite like one structure.

The notes which follow are not exhaustive but are intended to be of practical assistance in the use of our key.

SYNOPTIC TABLE OF PHILIPPINE ANOPHELES

The following classification of Philippine *Anopheles* is based largely on Christophers.(8) Following him, the local species may be classified as follows:

A. Tribe ANOPHELINI.

Genus I. *Chagasia* (none in the Philippine Islands).
Genus II. *Bironella* (none in the Philippine Islands).
Genus III. *Anopheles*.

B. Genus ANOPHELES.

Subgenus I. *Stethomyia* (none in the Philippine Islands).
Subgenus II. *Anopheles.*
Subgenus III. *Nyssorhynchus* (none in the Philippine Islands).
Subgenus IV. *Myzomyia.*

C. Subgenus ANOPHELES.

Group I. *Anopheles.*
Group II. *Arribalzagia* (none in the Philippine Islands).
Group III. *Christya* (none in the Philippine Islands).

D. Group ANOPHELES.

Series I. *Anopheles.*

1. *Anopheles aitkeni* var. *bengalensis.*
2. *Anopheles gigas* var. *formosus.*
3. *Anopheles insulæflorum.*
4. *Anopheles lindesayi* var. *benguetensis.*

Series II. *Lophoscelomyia* (none in the Philippine Islands).
Series III. *Myzorhynchus.*

5. *Anopheles baezai* (variety ?).
6. *Anopheles barbirostris.*
7. *Anopheles hyrcanus* var. *nigerrimus.*
8. *Anopheles hyrcanus* var. *sinensis.*
9. *Anopheles pseudobarbirostris.*

E. Subgenus MYZOMYIA.

Group I. *Neomyzomyia.*
Group II. *Myzomyia.*
Group III. *Pseudomyzomyia.*
Group IV. *Paramyzomyia* (none in the Philippine Islands).
Group V. *Neocellia.*
Group VI. *Cellia* (none in the Philippine Islands).

F. Group NEOMYZOMYIA.

10. *Anopheles kochi.*
11. *Anopheles kolambuganensis* (not included by Christophers).
12. *Anopheles leucosphyrus.*
13. *Anopheles tessellatus.*

G. Group MYZOMYIA.

14. *Anopheles filipinæ.*
15. *Anopheles mangyanus.*
16. *Anopheles minimus* var. *flavirostris.*

H. Group PSEUDOMYZOMYIA.

17. *Anopheles litoralis.*
18. *Anopheles ludlowi.*
19. *Anopheles parangensis.*
20. *Anopheles subpictus* var. *indefinitus.*
21. *Anopheles vagus* var. *limosus.*

I. Group NEOCELLIA.

22. *Anopheles annularis* (*fuliginosus*).
23. *Anopheles karwari.*
24. *Anopheles maculatus.*
25. *Anopheles philippinensis.*

The list of Philippine anophelines is provisional. Several species are still under scrutiny by King and also by Baisas. The table is the best we can prepare at present.

A. Subgenus ANOPHELES

I. Group ANOPHELES Series ANOPHELES

1. ANOPHELES AITKENI var. BENGALENSIS Puri, 1930.

Stethomyia pallida Ludlow, 1905, belongs either to this variety or to *A. insulæflorum.* Only a single female was described.
A. X-2 of Baisas, 1927.

2. ANOPHELES GIGAS var. FORMOSUS Ludlow, 1909.

3. ANOPHELES INSULÆFLORUM Swellengrebel and Swellengrebel de Graaf, 1920.

Stethomyia aitkeni var. *insulæflorum* of Swellengrebel, 1920.
A. X-3 of Baisas, 1927.

4. ANOPHELES LINDESAYI var. BENGUETENSIS King, 1931.

II. Group ANOPHELES Series MYZORHYNCHUS

5. ANOPHELES BAEZAI Gater, 1933. (?).

A. umbrosus Theobald of Mieldazis, 1930, and other Philippine authors.
A. umbrosus var. *separatus* Leicester of Philippine authors.

We are not certain that our form is true *baezai.* It may be what Gater calls "form A." It is certainly not typical *umbrosus.*

6. ANOPHELES BARBIROSTRIS Van der Wulp, 1884.

? *vanus* Walker, 1860.
Myzorhynchus vanus of Ludlow, 1908.
A. barbirostris varieties 1 and 2 (in part) of Baisas, 1927.

Some larvæ, collected in 1933 by Malaria Investigations in Palawan, may be a new variety of *barbirostris.* They show much less branching of the clypeal hairs.

7. ANOPHELES HYRCANUS var. NIGERRIMUS Giles, 1900.

? *nero* Doleschall, 1851.
A. indiensis Theobald, 1901.
? *pursati* Laveran, 1902.
Anopheles II of Schüffner, 1902 (probably).
A. bentleyi Bentley, 1902.
Myzorhynchus minutus Theobald, 1903.
Myzorhynchus argyropus Swellengrebel, 1914.
Myzorhynchus peditaeniatus Leicester, 1908, of Walker and Barber, 1914 (in part).

8. ANOPHELES HYRCANUS var. SINENSIS Wiedemann, 1828.

A. plumiger Dönitz, 1901.
A. jeosoensis Tsuzuki, 1902 (*A. vesoensis* 1901).
Myzorhynchus peditaeniatus Leicester, 1908, of Walker and Barber, 1914 (in part).
A. hyrcanus varieties 1 and 2, Baisas, 1929 (in part).
A. sinensis of Philippine authors.

9. ANOPHELES PSEUDOBARBIROSTRIS Ludlow, 1902.

A. barbirostris var. No. 1, Baisas, 1927 (in part).
A variety intermediate between *barbirostris* and *hyrcanus*, Baisas, 1927.

B. Subgenus MYZOMYIA

I. Group NEOMYZOMYIA

10. ANOPHELES KOCHI Dönitz, 1901.

A. ocellatus Theobald, 1901.
A. Ia of Schüffner, 1902.
Cellia flava Ludlow, 1908.
Christophersia halli James, 1910.
N. tessellata of Mathis and Leger, 1910.

11. ANOPHELES KOLAMBUGANENSIS Baisas, 1931.

Unnamed species of Manalang, 1929.
A. kolambuganensis, Manalang MSS (Baisas, 1931).

12. ANOPHELES LEUCOSPHYRUS Dönitz, 1901.

Myzomyia ? elegans James, 1903.
(Note: The *leucosphyrus* of Luzon, those of Mindanao, and the Balabac forms, may be geographic variants of one species. The "*near-leucosphyrus*" of Mindanao appears to be a distinct species.)

13. ANOPHELES TESSELLATUS Theobald, 1901.

A. deceptor Dönitz, 1902.
Myzomyia thorntonii Ludlow, 1904.
Dactylomyia ceylonica Newstead and Carter, 1910.
A. punctulatus of James and Liston, 1910.
A. X–6 of Baisas, 1927.

II. Group MYZOMYIA

14. ANOPHELES FILIPINÆ Manalang, 1930 (Christophers and Puri, 1931).

A. minimus varieties 1, 2, and 3 of Baisas, 1927.
A. aconitus var. *filipinæ* Manalang, 1930.
A. "minimus variety" of Philippine authors.

15. ANOPHELES MANGYANUS Banks, 1907.

Perhaps *Myzomyia funesta* of Whitmore, 1904, in part.
? *Pyretophorus pitchfordi* Giles, 1904.
Myzomyia mangyana Banks, 1907.
A. christophersi Edwards (in part, not Theobald) 1914.
Myzomyia febrifera Banks, 1914.
Anopheles febrifer Walker and Barber, 1914 (perhaps in part).

Anopheles (*Myzomyia*) *christophersia* Ludlow (in part, not Theobald), 1915.

A. minimus Christophers (in part, not Theobald), 1916.

Probably *A. minimus* and *A. funestus* (in part) and possibly *A. aconitus* var. *filipinæ* (in part) of Philippine authors.

16. ANOPHELES MINIMUS var. FLAVIROSTRIS Ludlow, 1914 (King, 1932).

Pyretophorus minimus Giles (not Theobald) 1904.

Myzomyia funesta of Whitmore (in part), 1904.

Myzomyia funesta Ludlow (not Giles) 1905 to 1914 (in part).

Very likely *A. febrifer* of Walker and Barber (1914) was in large part this species.

Myzomyia flavirostris Ludlow 1914.

A. christophersi Edwards (in part, not Theobald), 1914.

Anopheles christophersi Ludlow (in part, not Theobald), 1915.

A. minimus Edwards (not Theobald), 1915.

A. minimus var. *aconitus* Christophers (not Dönitz), 1916.

A. (*Myzomyia*) *minimus* Christophers (in part, not Theobald), 1924.

A. minima var. *flavirostris* Yamada, 1925.

A. minimus Baisas, 1927 (description of larva).

A. funestus Manalang (in part, not Giles), 1930, et seq.

III. Group PSEUDOMYZOMYIA

17. ANOPHELES LITORALIS King, 1932.

Myzomyia ludlowi Theobald 1903 in part as used by Philippine authors.

A. sundaicus of Rodenwaldt, 1926 (in part perhaps).

This is the "salt-water" *A. ludlowi* of Philippine authors.

18. ANOPHELES LUDLOWI Theobald, 1903.

Myzomyia ludlowi Theobald, 1903.

M. vaga of Schüffner and Swellengrebel, 1917.

A. hatorii of Koidzumi, 1923 (in part perhaps).

A. (*Myzomyia*) *ludlowi* var. *flavescens* of Swellengrebel, 1921.

This is the "fresh-water" *A. ludlowi* of Philippine authors.

19. ANOPHELES PARANGENSIS Ludlow, 1914.

M. ludlowi variety of Ludlow, 1914.

Myzomyia parangensis Ludlow, 1914.

20. ANOPHELES SUBPICTUS var. INDEFINITUS Ludlow, 1904.

A. formosaensis II (in part) Tsuzuki, 1902.

Myzomyia rossii Giles var. *indefinita* Ludlow, 1904.

A. subpictus Grassi of Tiedemann, 1927.

A. subpictus var. *malayensis* Hacker of Tiedemann (in part), 1927.

A. rossii (river-slew type) Baisas, 1927.

21. ANOPHELES VAGUS var. LIMOSUS King, 1932.

A. formosaensis II Tsuzuki (in part), 1902.

A. subpictus var. *malayensis* (in part) of Tiedemann, 1927.

A. rossii (pool type) Baisas, 1927.

A. rossii Giles (in part) of Philippine authors.
A. vagus of Philippine authors.

IV. Group NEOCELLIA

22. ANOPHELES ANNULARIS Van der Wulp, 1884.

A. fuliginosus Giles, 1900.
A. leucopus Dönitz, 1901.
A. jamesi Liston, 1901.
A. nagpori James and Liston, 1904.
Nyssorhynchus fuliginosus var. *adiei* James and Liston, 1911.

23. ANOPHELES KARWARI James, 1903.

Nyssorhynchus karwari James, 1903.
A. nigrans Stanton, 1912.

24. ANOPHELES MACULATUS Theobald, 1901.

A. maculata Theobald, 1901.
? *Nyssorhynchus theobaldi* of Ludlow, 1901.
Nyssorhynchus pseudowillmori Theobald, 1910.
A. maculatus var. *dravidicus* Christophers, 1924.
A. hanabusai Yamada, 1925.

We have not run across an aberrant form of *A. maculatus* which, according to Christophers, 1931, resembles *N. theobaldi* in form. The few *A. maculatus* we have from Baguio (5,000 feet altitude) show, on the average, as much scaling on the dorsum of the abdomen of the adults as those from the lowlands of Luzon, but less than those from Mindanao. None, however, shows any maculation on the palps, while a few from the lowlands have this characteristic.

25. ANOPHELES PHILIPPINENSIS Ludlow, 1902.

Nyssorhynchus nivipes Theobald, 1903.
A. pallidus (in part) Dyar and Shannon (not Theobald), 1925.
Pyretophorus freeræ Banks, 1906.
A. pampangensis Brunetti, 1920.
A. fuliginosus of Stanton, 1915.

C. Undetermined

26. Balabac ANOPHELES of undetermined species or variety (?).

Some larvæ of a species or variety not yet reported in the Philippines were found in Balabac, Palawan, by P. F. Russell and Andres M. Nono. Similar larvæ have been found recently in Iwahig, Palawan. These are being studied and as soon as possible a report will be made. Possibly this anopheline is a variety of *leucosphyrus*.

27. ANOPHELES near-LEUCOSPHYRUS (?).

Taken in Mindanao by F. E. Baisas and D. Santiago.

28. ANOPHELES of undetermined variety (?).

A larva apparently belonging to the *aitkeni* group but having the inner anterior clypeal with nine and ten main branches each (not the fine sub-branches found in some *bengalensis*), was found on Mount Banahao by F. E. Baisas and D. Santiago.

DESCRIPTIVE NOTES

We have used the following abbreviations in our notes:

i. c. Inner clypeal hairs.
o. c. Outer clypeal hairs.
p. c. Posterior clypeal hairs.
i. o. Inner occipital hairs.
o. o. Outer occipital hairs.
i. s. p. Inner submedian prothoracic hairs.
m. s. p. Middle submedian prothoracic hairs.
sh. Shoulder hairs (i. s. p. and m. s. p. included).
p. v. s. m. Prothoracic ventral submedian or prothoracic hair 13.
p. t. p. Prothoracic pleural hair group.
m. t. 5. Mesothoracic hair 5.
m. t. p. Mesothoracic pleural hair group.
m. t. v. s. m. Metathoracic ventral submedian or metathoracic hair 13.
t. pal. Thoracic palmate.
abd. pal. Abdominal palmate.
a. pal. Ante- or prepalmate.
lat. Lateral hairs.
d.-l. p. Dorsolateral posterior (hair 5 on segment VI).
ter. pl. Tergal plates.
pect. teeth. Short and long teeth of pecten.
stig. cl. Stigmal club.

Roman numerals refer to abdominal segments. Arabic numbers in the notes below refer to the numbers of branches or leaflets of a given hair.

1. ANOPHELES AITKENI var. BENGALENSIS. Plate 2; Plate 29, figs. A and B; Plate 32, fig. 3; Plate 33, fig. 9.

A small dark larva; i. c. closely approximated, branched at about middle into 2 to 4 main branches with or without fine subbranches; o. c. 2 or 3 lateral branches (rarely simple); p. c. 4 to 6; i. o. 4 to 6; o. o. 5 to 9; i. s. p. 1 to 13; m. s. p. 9 to 15; t. pal. developed, with 13 to 18 leaflets; abd. pal. I developed but small, with 7 to 16 leaflets; abd. pal. II to VII well developed, with 12 to 20 leaflets; lat. III numerous branches, feathery; lat. IV and V 4 to 7.

The types of branching of the clypeal hairs which have been illustrated by Puri (7) and Gater (18) are also found in the Philippines. We found one specimen on Mount Banahao, at an elevation of about 3,000 feet, whose i. c. are branched 9 or 10. One of the o. c. has 7. (The other o. c. is missing.) At present we prefer to group our varieties under this one heading of *aitkeni* var. *bengalensis*, although in the key we have noted the variety from Banahao.

2. ANOPHELES GIGAS var. FORMOSUS. Plate 7.

Largest local anopheline larva. Light yellow to gray. Rarely has distinct white markings on body. I. c. closely approximated usually simple, sometimes 2 or 3; o. c. simple or 2 to 6 lateral branches; p. c. relatively long with 2 to 8; i. o. 4 to 12; o. o. 6 to 16; i. s. p. relatively small, 3 to 10; m. s. p. 8 to 15; t. pal. rudimentary; abd. pal. I and II rudimentary; abd. pal. III to VII with leaflets smoot or serrated, and unfilamented; lat. IV and V long, 2 to 5, usually 3 or 4; lat. VI very short.

3. ANOPHELES INSULÆFLORUM. Plate 10; Plate 33, fig. 10.

A medium-sized larva, usually very black; i. c. closely approximated, simple; o. c. short, simple, sometimes forked; p. c. 3 to 6; i. o. 3 to 6; o. o. 4 to 10; sh. short and stout, the inner sometimes much like an elongated fan in shape, and usually 3 to 11 stout branches, rarely simple, middle hair 8 to 14; t. pal. developed; abd. pal. all developed.

4. ANOPHELES LINDESAYI var. BENGUETENSIS. Plate 15; Plate 30, figs. C and D.

A larva of more than medium size, easily recognized by alternation of white and black markings on its body; white markings fairly well retained in formalin. (Some larvæ collected at Haight's Place at 7,500 feet have imperfect or no white markings.) I. c. closely approximated, long, simple, rarely forked; o. c. simple; p. c. 2 to 5, sometimes simple; i. o. usually simple, sometimes forked; o. o. 2 to 6 rarely simple; i. s. p. 10 to 16; m. s. p. 8 to 15; t. pal. developed; abd. pal. I rudimentary; abd. pal. II to VII developed, leaflets filamented; lat. IV and V 2 or 3; lat. VI very short.

5. ANOPHELES BAEZAI (?) Plate 3; Plate 31, fig. 5.

A large brown to black larva, only the young forms have definite white markings; i. c. closely approximated, finely branched distally; o. c. dichotomously branched, less plumose than in any of the *barbirostris-hyrcanus* group; p. c. simple or forked; i. o. 2 to 5; o. o. 2 to 5; i. s. p. 3 to 6 apically; m. s. p. 6 to 10; t. pal. rudimentary, branched like ordinary hair with 4 to 6; abd. pal. no true palmates but instead there are ordinary hairs branched 7 to 15; lat. IV 7 to 12; lat. V 2 or 3, sometimes simple. One of the bladelike projections at the distal end of the antenna is not pointed but truncated and is notched at its tip. (Note: Certain adult characters indicate that this larva may be a variety and not true *baezai*.)

6. ANOPHELES BARBIROSTRIS. Plate 4; Plate 30, fig. A.

One of largest larvæ, varying in color from dark brown to grayish yellow, with or without white markings of various patterns; i. c. closely approximated, simple, rarely forked; o. c. dichotomously branched, more than 20, plumose; p. c. usually branched; i. o. 5 to 16; o. o. 6 to 15; i. s. p. 4 to 15 near base; m. s. p. 9 to 19; t. pal. developed but not pigmented; abd. pal. all developed; I and II not pigmented; lat. VI very short; d.-l. p. present; stig. cl. absent.

A few specimens of "*barbirostris*" in our collection, from Iwahig, Palawan, have less than the usual branching of the outer clypeal hair and it is possible that a separation will have to be made between the larvæ having more than 20 branches—*A. barbirostris*—and those having less than 20 branches. The latter may be *A. barbumbrosus* Strickland and Choudhury, 1927, as described by Gater, 1934, but we do not have enough adults to make a definite pronouncement.

7. ANOPHELES HYRCANUS var. NIGERRIMUS. Plate 8.

A large larva, like *barbirostris*, light yellow, yellow-red to dark brown; i. c. closely approximated, usually simple, sometimes 2 to 7; o. c. dichotomously branched, branches long, more than 10, plumose; p. c. 2 to 8 (rarely simple); i. o. 3 to 10; o. o. 2 to 8; i. s. p. single or apically branched 2 or 3; p. v. s. m. short stem, 4 or 5, branches not widely separated; m. t. 5 small, with slender curved branches 5 to 16, usually 6 to 8; m. t. v. s. m. 2 to 4, usually 3 (rarely simple); t. pal. well developed, not pigmented; abd. pal. well developed, I and II not pigmented; lat. IV and V 2 or 3 (rarely simple); lat. VI very short; d.-l. p. present; stig. cl. absent.

8. ANOPHELES HYRCANUS var. SINENSIS. Plate 9; Plate 32, fig. 8.

Somewhat smaller than *nigerrimus* but resembling it closely in coloration. This larva is similar to *nigerrimus* in most of its hairs, but presents the following differences:

P. v. s. m. a long stem with usually more than 5 branches. These branches are spread out, relatively far apart, along the main rachis. In *nigerrimus* the stem is short and the 4 or 5 branches originate close together near apex of main rachis. Rarely this hair in *sinensis* resembles that in *nigerrimus*.

M. t. 5 in *sinensis* is always stout, straight, and with straight branches 2 to 5, usually 3 or 4. In *nigerrimus* this hair is

shorter, slenderer, and with slender, curving branches, spread out in a starlike pattern.

M. t. v. s. m. simple or branched 2 to 4, usually 2; in *nigerrimus* the usual branching is 3.

In *sinensis* larvæ from Baguio (5,000 feet) the prothoracic hair 13 (p. v. s. m.) tends to have fewer branches than in lowland *sinensis* and it has a pattern intermediate between the usual *sinensis* and *nigerrimus*. Mesothoracic hair 5 (m. t. 5) gives an accurate index in having straight stout branches like the lowland *sinensis*, although there are usually 4 and sometimes 5 branches in the Baguio larvæ. The metathoracic hair 13 (m. v. s. m.) usually has 3 branches instead of 2, as in the lowland species.

9. ANOPHELES PSEUDOBARBIROSTRIS. Plate 23; Plate 33, fig. 11.

One of the largest of local anopheline larvæ. Varies in color from black to light brown, with or without markings of indefinite pattern; i. c. finely branched at about apical third, closely approximated; o. c. dichotomously branched, plumose; p. c. short, simple, sometimes branched; i. o. 6 to 13; o. o. 2 to 11; i. s. p. simple or branched 2 to 4 at middle; m. s. p. 8 to 17; abd. pal. developed; lat. IV to VI all long and branched. (This is the only species of that group of local anopheline larvæ having closely approximated i. c. in which the lat. VI is long and well developed.) Stig. cl. present.

10. ANOPHELES KOCHI. Plate 12; Plate 33, fig. 3.

A medium-sized larva, light brown with a distinct white spot on the anterior half of thorax and another on tip of abdomen; i. c. finely branched, widely separated; o. c. simple; p. c. short, simple, occasionally forked; i. o. simple or forked; o. o. simple or forked, sometimes 3-branched; i. s. p. slender 5 to 12; m. s. p. 8 to 15; sh. slender stem and branches, tubercle small, not confluent; p. v. s. m. 3 to 6 slender branches; t. pal. developed, leaflets not well spread; abd. pal. I rudimentary; abd. pal. II developed, not pigmented; abd. pal. III–VII well developed and pigmented; lat. III up to 11 usually less than 10 branches; lat. IV to VI 2 to 3, sometimes single, IV and V more than half length of III; ter. pl. small.

11. ANOPHELES KOLAMBUGANENSIS. Plate 13.

A medium-sized larva easily recognized because of its very white and black markings, the white being sharply retained for some years in formalin or Gater's fluid; i. c. finely branched, widely separated; o. c. short, simple or finely branched; p. c.

2 to 8; i. o. simple or 2 or 3; o. o. 2 to 7; sh. stout, dark, bases confluent, inner 12 to 26, middle 8 to 15; t. pal. developed; abd. pal. I rudimentary; abd. pal. II to VII developed; lat. IV and V 4 to 5, half or less the length of III; lat. VI very short; ter. pl. small; ant. half of thorax, segments II, V, and VIII and sometimes VII totally white.

12. ANOPHELES LEUCOSPHYRUS. Plates 14 and 28; Plate 29, fig. F; Plate 31, fig. 4; Plate 32, figs. 5 and 10.

A medium-sized, light brown larva; i. c. finely branched, widely separated; o. c. simple; p. c. simple or forked; i. o. simple or branched 2 or 3; o. o. simple or branched 2 or 3; sh. fairly stout, bases confluent, inner 9 to 19, middle 8 to 15; t. pal. rudimentary; abd. pal. I and II rudimentary; abd. pal. III to VII developed, leaflets filamented and markedly serrated; lat. III more than 10 branches; lat. IV to VII about equal length 2 or 3; ter. pl. small.

13. ANOPHELES TESSELLATUS. Plate 25; Plate 32, figs. 1, 3, and 7.

A medium-sized, light to dark brown larva; i. c. finely branched, widely separated; o. c. very short, simple; p. c. very short, simple, rarely forked; i. o. 2 to 4, rarely simple; o. o. 2 to 4; sh. small, inner 3 to 6; middle 7 to 12; t. pal. fairly well developed; abd. pal. I and II rudimentary; abd. pal. III to VII developed, not pigmented, not filamented, not serrated or only faintly so; lat. III less than 10 branches; lat. IV to VI 2 or 3, rarely simple; ter. pl. small.

14. ANOPHELES FILIPINÆ. Plate 5; Plate 33, fig. 8.

A small light brown or yellowish to dark brown larva; i. c. widely separated, finely branched; o. c. simple or branched laterally, branches few and short; i. o. 2 to 8; o. o. 2 to 8; i. s. p. 12 to 22 (branched less than either *mangyanus* or *minimus* var. *flavirostris*); m. s. p. 10 to 18; t. pal. developed; leaflets taper into long pointed filaments; abd. pal. all developed; a. pal. II and VII usually branched near apex; ter. pl. large, the one on II not concave or notched.

15. ANOPHELES MANGYANUS. Plate 19; Plate 33, fig. 7.

A small very dark larva; i. c. simple, widely separated; o. c. simple; p. c. usually simple, rarely forked; i. o. 2 to 9; o. o. 3 to 10; sh. stout, dark; inner 15 to 26; middle 12 to 20; t. pal. developed, with leaflets extending into slender, very fine points; a. pal. VII simple, or forked distally; ter. pl. all large; II concave, IV to VII much rounded or blunt at the edges.

16. ANOPHELES MINIMUS var. FLAVIROSTRIS. Plates 1 and 20; Plate 33, fig. 6.

A small dark larva; i. c. simple, long, widely separated; o. c. usually simple, occasionally 2 or 3; p. c. usually simple, occasionally 2 or 3; i. o. 2 to 12; o. o. 2 to 13; sh. stout, dark; inner 18 to 30, middle 11 to 20; t. pal. developed, the leaflets not extended into slender fine points; abd. pal. I to VII all developed; a pal. VII branched basally; ter. pl. all large; II concave posteriorly; IV to VII tapering towards edges.

17. ANOPHELES LITORALIS. Plate 16.

A medium-sized dark green to gray larva; i. c. simple, widely separated; o. c. simple, and long, sometimes nearly as long as the i. c.; p. c. long and simple; i. o. simple, rarely branched; o. o. 2 to 5 rarely simple; sh. inner 2 to 11; middle 2 to 10; t. pal. rudimentary; abd. pal. I rudimentary; abd. pal. II to VII developed; lat. IV to VI 2 or 3, branches originating nearer the base than those of *ludlowi;* pec. teeth not markedly different.

18. ANOPHELES LUDLOWI. Plate 17.

A medium-sized gray-brown larva; i. c. simple, widely separated; o. c. simple, over half the length of i. c.; p. c. simple: i. o. simple, rarely branched; o. o. 2 to 7; sh. inner 8 to 16, middle 8 to 14; t. pal. rudimentary; abd. pal. I rudimentary; abd. pal. II to VII developed; lat. IV to VI 4 or 5; the branches of IV and V are farther away from the base than those of *litoralis.*

19. ANOPHELES PARANGENSIS. Plate 21; Plate 31, fig. 2; Plate 33, fig. 4.

A medium-sized brown to gray larva; i. c. simple, widely separated; o. c. simple, rarely forked; p. c. simple; i. o. simple, rarely forked; o. o. 3 to 5; sh. typically more branches than *subpictus* var. *indefinitus;* inner 12 to 20; middle 12 to 19; t. pal. rudimentary; p. t. p. and m. t. p. each of these pleural hair groups has a feathered hair, unlike those of any other species of the *ludlowi-rossi* group; the other species may have a hair branched, at the most 2 or 4; abd. pal. I developed; leaflets usually broader and more widely spread than those of *subpictus* var. *indefinitus;* abd. pal. II to VII developed; lat. IV to VI 2 to 4 usually 3, near the base like *subpictus* var. *indefinitus;* ter. pl. small.

20. ANOPHELES SUBPICTUS var. INDEFINITUS. Plate 24; Plate 29, fig. C; Plate 32, figs. 4, 6, and 11; text figs. 1 to 4.

A medium-sized brown to gray larva; may have small white spots on thorax and abdomen; i. c. simple, widely separated; o. c. simple, more than half the length of i. c.; p. c. simple or forked, more than half the length of i. c.; i. o. simple, rarely

2 or 3; o. o. 3 to 7; p. t. p. no feathered hair; m. t. p. no feathered hair; sh. inner 7 to 16, rarely (as in Mindanao) 4 or 5, middle 6 to 16; t. pal. rudimentary; abd. pal. I to VII developed; lat. IV to VI 3; ter. pl. small.

21. ANOPHELES VAGUS var. LIMOSUS. Plate 26; Plate 32, fig. 9; Plate 33, fig. 1.

A medium-sized brownish to gray larva; i. c. simple (rarely forked), widely separated; o. c. simple, half or less length of i. c.; p. c. simple, relatively shorter than others of *ludlowi-rossi* group; i. o. simple, rarely branched; o. o. 3 to 9; sh. inner 10 to 18; middle 11 to 17; t. pal. rudimentary; abd. pal. I rudimentary; abd. pal. II to VII developed, leaflets generally narrower and not so spread out as those of *indefinitus* and *ludlowi;* lat. IV to VI usually 2 branches near base; pec. teeth short and long, markedly different.

22. ANOPHELES ANNULARIS (FULIGINOSUS). Plate 6; Plate 30, fig. B.

A medium-sized dark green to gray larva; no white markings on fresh specimens; i. c. widely separated, many fine branches on distal two-thirds; o. c. dichotomously branched; branches numerous and long; plumose; p. c. 2 to 5 (rarely simple); i. o. simple or forked; o. o. 3 to 7; i. s. p. stout, pigmented, bases confluent, inner 19 to 26; m. s. p. 12 to 18; t. pal. well developed; lat. IV to VI 2 to 5.

23. ANOPHELES KARWARI. Plate 11; Plate 29, fig. D; Plate 31, fig. 1; Plate 33, fig. 5.

A medium-sized greenish gray larva; i. c. finely branched, widely separated; o. c. finely branched laterally, branches short; p. c. simple and short; i. o. simple or branched; o. o. branched up to 5; sh. stout; inner 20 to 25; middle 17 to 21; p. v. s. m. 3 to 6 slender branches; t. pal. rudimentary; abd. pal. I rudimentary; abd. pal. II fairly well developed; abd. pal. III to VII well developed; filaments of leaflets short and knob-ended; lat. IV to VI 5 to 9, usually 7 (this is usually more than *maculatus*); IV and V are more than half the length of III; ter. pl. small.

24. ANOPHELES MACULATUS. Plate 18; Plate 29, fig. E.

A medium-sized brownish to light gray larva; i. c. finely branched, widely separated; o. c. usually finely branched, sometimes simple; p. c. simple, short; i. o. simple; o. o. 2 to 5; p. v. s. m. 3 to 6 slender branches; sh. stout, inner 14 to 21, middle 14 to 19; t. pal. rudimentary; abd. pal. I rudimentary; abd. pal. II to VII developed with broad leaflets, the filaments of which are long and pointed; lat. IV to VI 5 to 7, IV and V more than half the length of III; ter. pl. small.

25. ANOPHELES PHILIPPINENSIS. Plate 22.

A medium-sized larva easily recognized by its white markings on the thorax and abdomen, in fresh specimen; i. c. many fine branches on anterior two-thirds, widely separated; o. c. branched dichotomously, branches long and numerous, plumose; p. c. 2 to 4; i. o. 2 to 5. In some countries *A. philippinensis* is differentiated from *A. annularis* (*fuliginosus*) on the basis that the i. o. of the former is branched while in the latter it is simple. This does not apply in the Philippines as this hair in our *annularis* (*fuliginosus*) is as often forked as it is simple. O. o. 3 to 5; sh. fairly stout, not pigmented. Bases not confluent, although this is not always clearly apparent. Rarely the bases of one or the other but not both are directly connected. A better criterion is the fact that the shoulder hairs of *philippinensis* are not pigmented, whereas those of *annularis* are always highly pigmented. This is especially useful in identifying mounted larval skins in which the position of the shoulder hairs is so distorted that the bases appear confluent, or else very widely separated. T. pal. developed; abd. pal. I to VII developed; lat. IV to VI 2 to 4, with branches more toward apex, generally, than those of *annularis* (*fuliginosus*).

26. Balabac species or variety (?). Plate 27; Plate 31, fig. 3; Plate 33, fig. 2.

Above medium size, light brown to brown; i. c. finely branched, widely separated; o. c. simple; p. c. simple, short; i. o. simple or forked; o. o. simple or forked; p. v. s. m. 3 to 6 slender branches; sh. stout but short, with large tubercle and confluent bases; inner 12 to 15, middle 10 to 11; t. pal. rudimentary; abd. pal. I rudimentary; abd. pal. II to VII developed; lat. III 11 to 15 or usually more branches; lat. IV and V 3, more than half length of lat. III; ter. pl. small.

27. ANOPHELES near-LEUCOSPHYRUS (?). Plate 28; Plate 31, fig. 4; Plate 32, fig. 5.

A medium-sized dark larva; i. c. widely separated, having more and coarser branches than any other member of the *leucosphyrus* group; o. c. over half the length of the inner, coarsely branched; p. c. longer than the outer, coarsely branched; i. o. usually simple, but may be forked; o. o. usually forked, but may be branched up to 4; rarely simple; p. v. s. m. 7 to 13 fairly stout branches; sh. inner 13 to 18; middle 10 to 16; t. pal. developed; abd. pal. I rudimentary; abd. pal. II to VII developed; lat. IV to VI long, 2 or 3 branched (usually 3).

It is possible that the Luzon *leucosphyrus*, the Mindanao *leucosphyrus*, and the "Balabac species or variety" are geographic

variants of the same species. "Near-*leucosphyrus*" appears to be a distinct species. Typically the Luzon specimens of *leucosphyrus* have rudimentary palmates II. This hair is more developed in the Mindanao form and is best developed in the Balabac larvæ. None seems to be *A. leucosphyrus* var. *hackeri* Edwards, 1921, as all have considerable variations from the typical larvæ of that species.

(The Balabac form, and also some taken at Iwahig, Palawan, have 9, 10, or 11 phallosomal leaflets in the adult males, the Mindanao form has up to 8, and the Luzon *leucosphyrus* has up to 7. The harpagonal spines of all three forms vary, being typically 2 but sometimes 3 or 4.)

Key to the Philippine species of the genus Anopheles.

1. Inner clypeal hairs closely approximated .... 2.
   Inner clypeal hairs widely separated .... 11.
2. Outer clypeal hairs simple or with short lateral branches (less than 10) .... 3.
   Outer clypeal hairs with long dichotomous branches (more than 10) 7.
3. Palmate I well developed .... 4.
   Palmate I not developed, hairlike .... 6.
4. Lateral hair III having few branches .... *insulæflorum*.
   Lateral hair III feathery .... 5.
5. Inner clypeal hairs having 2 to 4 main branches with or without fine subbranches .... *aitkeni* var. *bengalensis*.
   Inner clypeal hairs having 9 to 10 main branches without fine subbranches .... Banahao variety.
6. Leaflets of abdominal palmate unfilamented; no distinct white markings on body of fresh specimen .... *gigas* var. *formosus*.
   Leaflets of abdominal palmate filamented; distinct white markings on body of fresh specimen .... *lindesayi* var. *benguetensis*.
7. No true abdominal palmates .... *baezai*(?).
   Abdominal palmates present .... 8.
8. Stigmal club present; lateral hair VI about as long as those of IV and V .... *pseudobarbirostris*.
   Stigmal club absent; lateral hair VI very short .... 9.
9. Inner anterior submedian thoracic hair having 4 to 15 basal branches. *barbirostris*.
   Inner anterior submedian thoracic hair simple or having 2 or 3 apical branches .... 10.
10. Mesothoracic hair 5 having usually 3 or 4 straight branches; prothoracic hair 13 having long stem and 8 to 10 widely separated branches .... *hyrcanus* var. *sinensis*.
    Mesothoracic hair 5 having 6 to 8 slender curving branches; prothoracic hair 13 having short stem and usually 4 to 6 slightly separated branches .... *hyrcanus* var. *nigerrimus*.
11. Inner and outer clypeal hairs simple .... 12.
    Inner clypeal hairs branched .... 18.

12. Palmate I developed .... 13.
Palmate I not developed.. 16.
13. Tergal plates large .... 14.
Tergal plates small .... 15.
14. Leaflets of thoracic palmate hairs extended into long slender filaments; antepalmate VII single or forked apically..................... *mangyanus.*
Leaflets of thoracic palmate hair not extended into long slender filaments; antepalmate VII branched basally.
minimus var. *flavirostris.*
15. Each of pro- and mesothoracic pleural hair groups has a feathered hair.
parangensis.
Neither pro- nor mesothoracic pleural hair group has a feathered hair.
subpictus var. *indefinitus.*
16. Lateral hairs IV, V, and VI having 4 or 5 branches not close to the base .... *ludlowi.*
Lateral hairs IV, V, and VI having 2 or 3 branches arising close to the base .... 17.
17. Short and long teeth of pecten markedly different; outer clypeal hair less than half the length of inner......................... *vagus* var. *limosus.*
Short and long teeth of pecten not markedly different; outer clypeal hair over half the length of the inner.................................... *litoralis.*
18. Outer clypeal hair simple or having a few short lateral branches........ 19.
Outer clypeal hair having numerous long dichotomous branches........ 27.
19. Tergal plates large; first abdominal palmate rudimentary.... .... 20.
20. Palmate II developed .... 21.
Palmate II undeveloped .... 26.
21. Lateral hairs IV and V half or less than half the length of lateral hair III; distinct white markings on body............... *kolambuganensis.*
Lateral hairs IV and V more than half the length of lateral hair III.
22.
22. Posterior clypeal very long and branched; prothoracic hair 13 having 7 to 13 stout branches.. near-*leucosphyrus.*
Posterior clypeal short and simple; prothoracic hair 13 having 3 to 6 slender branches .... 23.
23. Lateral hairs IV to VI having 2 or 3 branches each.......................... 24.
Lateral hairs IV to VI having 4 to 9 branches each............................ 25.
24. Shoulder hairs with slender stem and branches; tubercles small and not confluent .... *kochi.*
Shoulder hairs with stout stem and branches; tubercles large and often confluent .... Balabac species or variety (?).
25. Filaments of abdominal palmates II to VII having short knobbed ends.
karwari.
Filaments of abdominal palmates II to VII having long pointed ends.
maculatus.
26. Leaflets of abdominal palmates III to VII filamented and markedly serrated. Lateral hair III having more than 10 branches.
leucosphyrus.
Leaflets of abdominal palmate III to VII not filamented and only slightly serrated if at all; lateral hair III having less than 10 branches.
tessellatus.

27. Bases of anterior submedian thoracic hairs confluent and pigmented; no white markings on fresh specimen........................ *annularis.*
Bases of anterior submedian thoracic hairs neither confluent nor pigmented; distinct white markings on fresh specimen. *philippinensis.*

SUMMARY

This paper presents a practical guide to the identification of the larvæ of Philippine anopheline mosquitoes. It consists essentially of five parts; namely, (*a*) a synoptic table, (*b*) a series of brief descriptive notes, (*c*) a dichotomous key, (*d*) the same key in wall-chart form (printed separately), and (*e*) a series of descriptive drawings; all have reference to fourth-stage larvæ of the local anophelines.

REFERENCES

1. LUDLOW, C. S. The mosquitoes of the Philippine Islands. Geo. Washington Univ. Wash. D. C. (1908) 1–65. See also War Dept. Bull. 4 Surg. Gen. Office (1914) 1–97. Numerous other references to Ludlow's works are given by Russell. (11)
2. BANKS, C. S. A list of Philippine Culicidae with descriptions of some new species. Philip. Journ Sci. 1 (1906) 977–1005. See other references to Banks given by Russell. (11)
3. MIELDAZIS, J. J. Reports to the International Health Board of The Rockefeller Foundation (unpublished) (1925–1928). Referred to by Russell. (11)
4. MANALANG, C. Morphology and classification of the Philippine variety of Anopheles aconitus Dönitz, 1902, and Anopheles minimus Theobald, 1901. Philip. Journ. Sci. 43 (1930) 247–261. Numerous other references given by Russell. (11)
5. BAISAS, F. E. Preliminary differential characters of Philippine anopheline mosquito larvae. Mon. Bull. Philip. Health Serv. 7 (1927) 267–280.

   The barbirostris-hyrcanus group of the Philippine Anopheles. Philip. Journ. Sci. 44 (1931) 425–448.
6. KING, W. V. The Philippine varieties of Anopheles gigas and Anopheles lindesayi. Philip. Journ. Sci. 46 (1931) 751–757.

   The Philippine Anopheles of the rossi-ludlowi group. Philip. Journ. Sci. 47 (1932) 305–342.

   Three Philippine Anopheles of the funestus-minimus subgroup. Philip. Journ. Sci. 48 (1932) 485–523.
7. PURI, I. M. Larvae of anopheline mosquitoes, with full description of those of the Indian species. Ind. Med. Res. Memoir 21 (1931) 99. Also Plate XIX. See also Ind. Journ. Med. Res. 16 (1928) 519–528.
8. CHRISTOPHERS, SIR S. R. Family Culicidae, Tribe Anophelini. Vol. IV. Diptera, in the Fauna of British India. Taylor and Francis, London (1933) 1–371.
9. MARTINI, E. Über einige für das System bedeutungsvolle Merkmale der Stechmücken. Zool. Jahrb. Abt. f. Syst. 46 (1923) 517–590.

10. ROOT, F. The larval pilotaxy of A. quadrimaculatus and A. punctipennis. Am. Journ. Hyg. 4 (1924) 710–724.

11. RUSSELL, P. F. Malaria and Culicidæ in the Philippine Islands: history and critical bibliography, 1898 to 1933. Dept. Agr. and Commerce, Manila. Technical Bull. 1 (1934) 1–109.

12. CHRISTOPHERS, S. R. The development and structure of the terminal abdominal segments, and hypopygium of the mosquito. Ind. Journ. Med. Res. 10 (1922–3) 538, 539, pl. 26.

13. IMMS, A. D. On the larval and pupal stages of Anopheles maculipennis, Meigen. Part II. The Larva. Parasitology 1 (1908) 107, pl. 9.

14. GATER, B. A. R. Notes on Malayan Mosquitoes III. The larval forms of Anopheles aitkeni James. Malayan Med. Journ. 8 (1933) 96–101. See also pp. 39–45, 180–189.

On Anopheles baďzai n. sp. from Malay peninsula. Bull. Raffles Mus. 8 (1933). See also this author's recently published Aids to identification of anopheline larvae in Malaya. Gov't. Printing Office, Singapore (1934) 1–160.

ILLUSTRATIONS

PLATES

The following numbers and letters apply to Plates 1 to 30. The descriptions of Plates 31 to 33 are given beyond. Only those parts are illustrated under each species which seem to be of practical use.

1. Clypeal hairs.
2. Antenna (showing antennal hair).
3. Shoulder hairs.
4. Thoracic palmate hair.
5. Mesothoracic hair 5.
6. Abdominal palmate hairs.
    a. Palmate hair I.
    b. Palmate hair II.
    c. Palmate hair IV.
7. Lateral hairs.
    a. Lateral hair III.
    b. Lateral hair IV.
    c. Lateral hair V.
    d. Lateral hair VI.
8. Pecten.
9. Tergal plates.
    a. Tergal plate II.
    b. Tergal plate V.
10. Prothoracic hair 13 (prothoracic ventral submedian).
11. Prothoracic hair 14 (subcervical).
12. Pleural hair groups.
    a. Prothoracic pleural hair group.
    b. Mesothoracic pleural hair group.
    c. Metathoracic pleural hair group.
13. Spiracular apparatus showing stigmal club.

14. *Anopheles leucosphyrus.*
15. *Anopheles lindesayi* var. *benguetensis.*
16. *Anopheles litoralis.*
17. *Anopheles ludlowi.*
18. *Anopheles maculatus.*
19. *Anopheles mangyanus.*
20. *Anopheles minimus* var. *flavirostris.*
21. *Anopheles parangensis.*
22. *Anopheles philippinensis.*
23. *Anopheles pseudobarbirostris.*
24. *Anopheles subpictus* var. *indefinitus.*
25. *Anopheles tessellatus.*
26. *Anopheles vagus* var. *limosus.*
27. Balabac species (?).
28. *Anopheles* near-*leucosphyrus* (?).
29. Variations in clypeal hairs.
    - A. *Anopheles aitkeni* var. *bengalensis* (?); from Mount Banahao.
    - B. *Anopheles aitkeni* var. *bengalensis;* with fine subbranches.
    - C. *Anopheles subpictus* var. *indefinitus;* unusual.
    - D. *Anopheles karwari;* finer branching.
    - E. *Anopheles maculatus;* very few branches.
    - F. *Anopheles leucosphyrus;* unusual branching.
30. Variations in clypeal hairs.
    - A. *Anopheles barbirostris;* from Balabac.
    - B. *Anopheles annularis;* branching of inner hair like those of *philippinensis.*
    - C. *Anopheles lindesayi* var. *benguetensis;* unusual inner clypeal hair.
    - D. *Anopheles lindesayi* var. *benguetensis;* fine branches on outer clypeal.
31. Pleural hair groups of some uncommon species.
    1. *Anopheles karwari.*
        - *a.* Prothoracic pleural hairs.
        - *b.* Mesothoracic pleural hairs.
    2. *Anopheles parangensis.*
        - *a.* Prothoracic pleural hairs.
        - *b.* Mesothoracic pleural hairs.
    3. Balabac species.
        - *a.* Prothoracic pleural hairs.
        - *b.* Mesothoracic pleural hairs.
        - *c.* Metathoracic pleural hairs.
    4. Near-*leucosphyrus.*
        - *a.* Prothoracic pleural hairs.
        - *b.* Mesothoracic pleural hairs.
        - *c.* Metathoracic pleural hairs.
    5. *Anopheles baezai* (?).
        - *a.* Prothoracic pleural hairs.
        - *b.* Mesothoracic pleural hairs.
        - *c.* Metathoracic pleural hairs.

32. Variations in shoulder and palmate hairs.
    1. *Anopheles tessellatus;* shoulder hairs.
    2. *Anopheles tessellatus;* shoulder hairs.
    3. *Anopheles aitkeni* var. *bengalensis;* shoulder hairs.
    4. *Anopheles subpictus* var. *indefinitus;* shoulder hairs.
    5. *Anopheles* near-*leucosphyrus* (?); palmate I.
    6. *Anopheles subpictus* var. *indefinitus;* palmate I, same specimen as 4.
    7. *Anopheles tessellatus;* palmate IV, slightly serrated.
    8. *Anopheles hyrcanus* var. *sinensis;* palmate IV, very narrow.
    9. *Anopheles vagus* var. *limosus;* palmate IV, very narrow.
    10. *Anopheles leucosphyrus;* palmate IV, very narrow, slightly serrated.
    11. *Anopheles subpictus* var. *indefinitus;* thoracic palmate.
33. Variations in pectens. Some lateral hairs III, tergal plates, and a stigmal club.
    1. *a* and *b* *Anopheles vagus* var. *limosus;* pectens.
    2. *a* and *b* Balabac species (?); pectens.
    3. *a* and *b* *Anopheles kochi;* pectens.
    4. *a* and *b* *Anopheles parangensis;* pectens, from one larva.
    5. *a* and *b* *Anopheles karwari;* pectens.
    6. *Anopheles minimus* var. *flavirostris;* tergal plates II and V.
    7. *Anopheles mangyanus;* tergal plates II and V.
    8. *Anopheles filipinæ;* tergal plates II and V.
    9. *Anopheles aitkeni* var. *bengalensis;* lateral hair III.
    10. *Anopheles insulæflorum;* lateral hair III.
    11. *Anopheles pseudobarbirostris;* stigmal plate, lateral view; *a*, stigmal club; *b*, spiracle.

TEXT FIGURES

FIG. 1. *Anopheles subpictus* var. *indefinitus;* head, camera lucida drawing, semidiagrammatic.
    2. Inner clypeal hair.
    3. Outer clypeal hair.
    4. Posterior clypeal hair.
    5 to 7. Frontal hairs.
    8. Inner occipital or sutural hair.
    9. Outer occipital or transutural hair.
    11. Antennal or shaft hair.

2. *Anopheles subpictus* var. *indefinitus;* dorsum of thorax, camera lucida drawing, semidiagrammatic, left half.
    A. Prothorax.
        1. Inner submedian prothoracic hair.
        2. Middle submedian prothoracic hair.
        3. Outer submedian prothoracic hair.
        4 to 7. Lateral prothoracic hairs.
    B. Mesothorax.
        5. This is one of the dorsolateral hairs 2 to 7.

C. Metathorax.

1. Thoracic palmate hair.

2 to 7. Dorsolateral hairs.

3. *Anopheles subpictus* var. *indefinitus;* venter of thorax, camera lucida drawing, semidiagrammatic, left half.

A. Prothorax.

9 to 12. Prothoracic pleural hair group.

13. Prothoracic ventral submedian hair.

B. Mesothorax.

9 to 12. Mesothoracic pleural hair group.

C. Metathorax.

9 to 12. Metathoracic pleural hairs.

13. Metathoracic ventral submedian hair.

4. *Anopheles subpictus* var. *indefinitus;* abdominal segments III to VII, dorsal view, camera lucida drawing, semidiagrammatic.

1. Palmate hair.

2. Prepalmate hair.

3 and 4. Small dorsolateral hairs.

5. Dorsolateral posterior hair.

6 and 7. Lateral hairs.

Ter. pl. Tergal plate.

5. Spiracular apparatus: *A. barbirostris* and *A. pseudobarbirostris.*

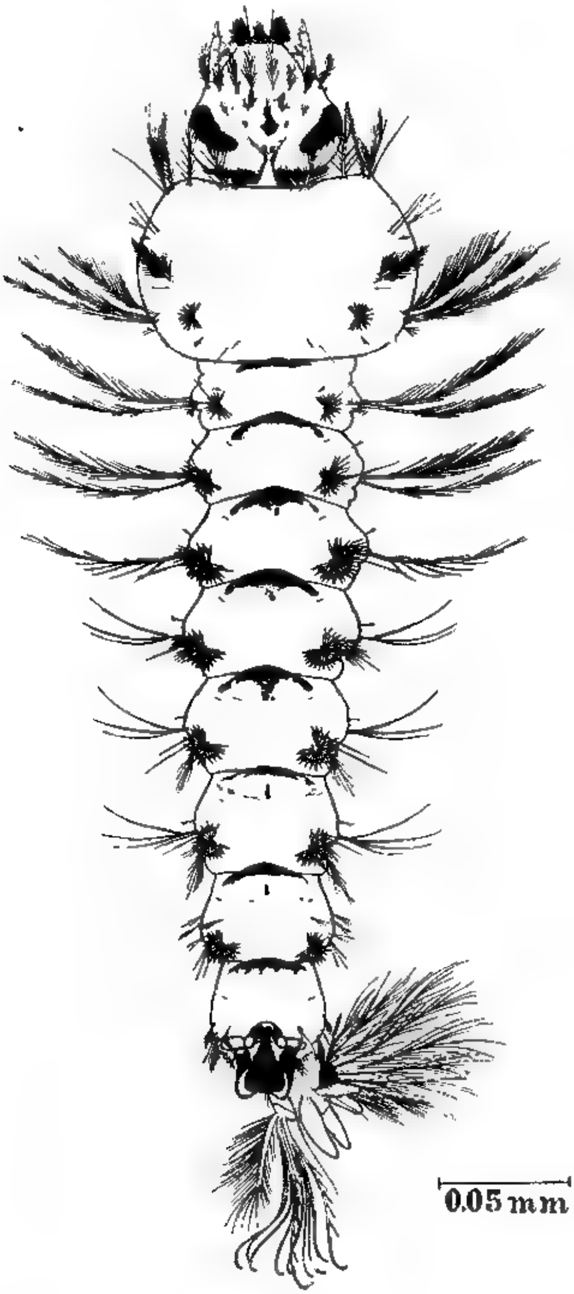

PLATE 1. ANOPHELES MINIMUS VAR. FLAVIROSTRIS.

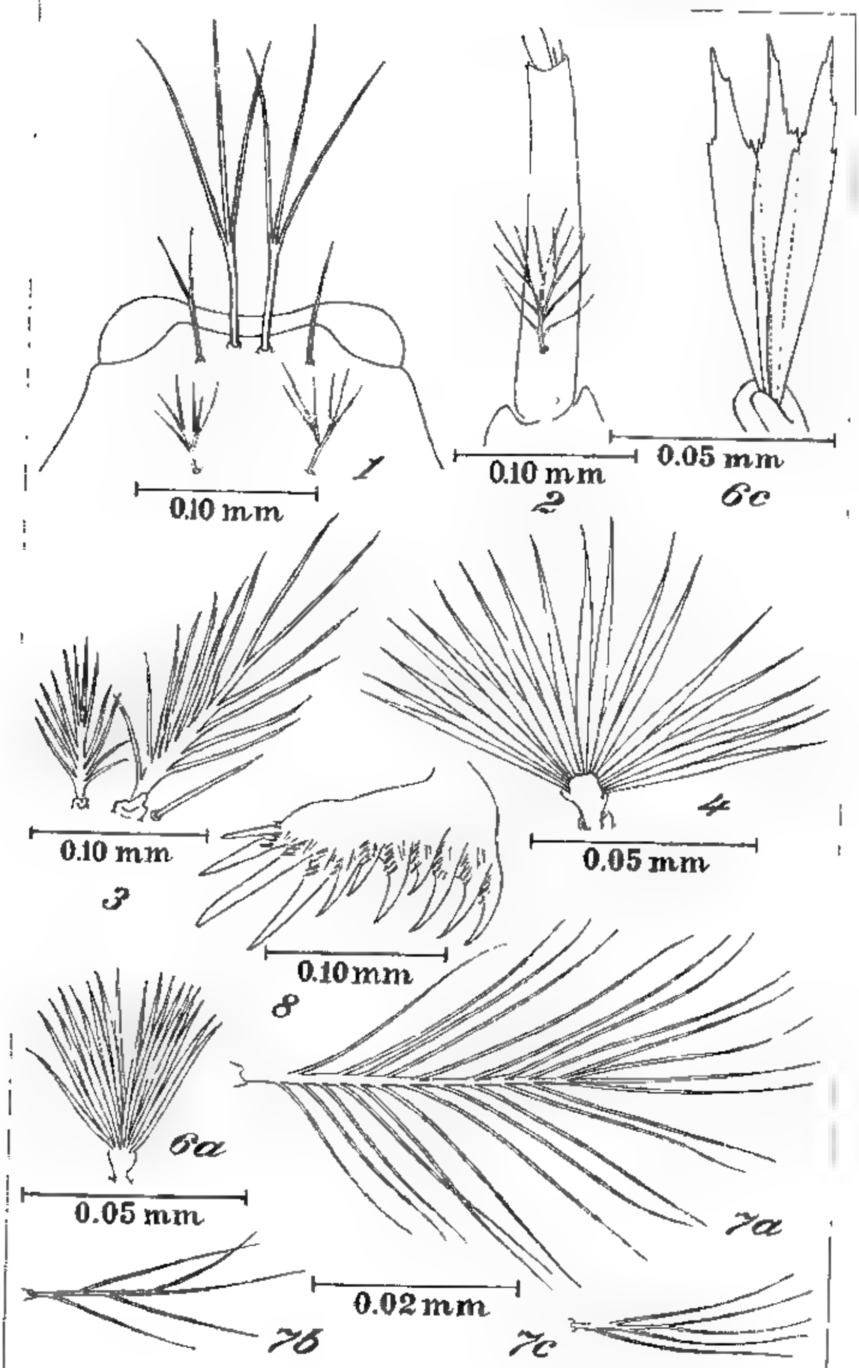

PLATE 2. ANOPHELES AITKENI VAR. BENGALENSIS.

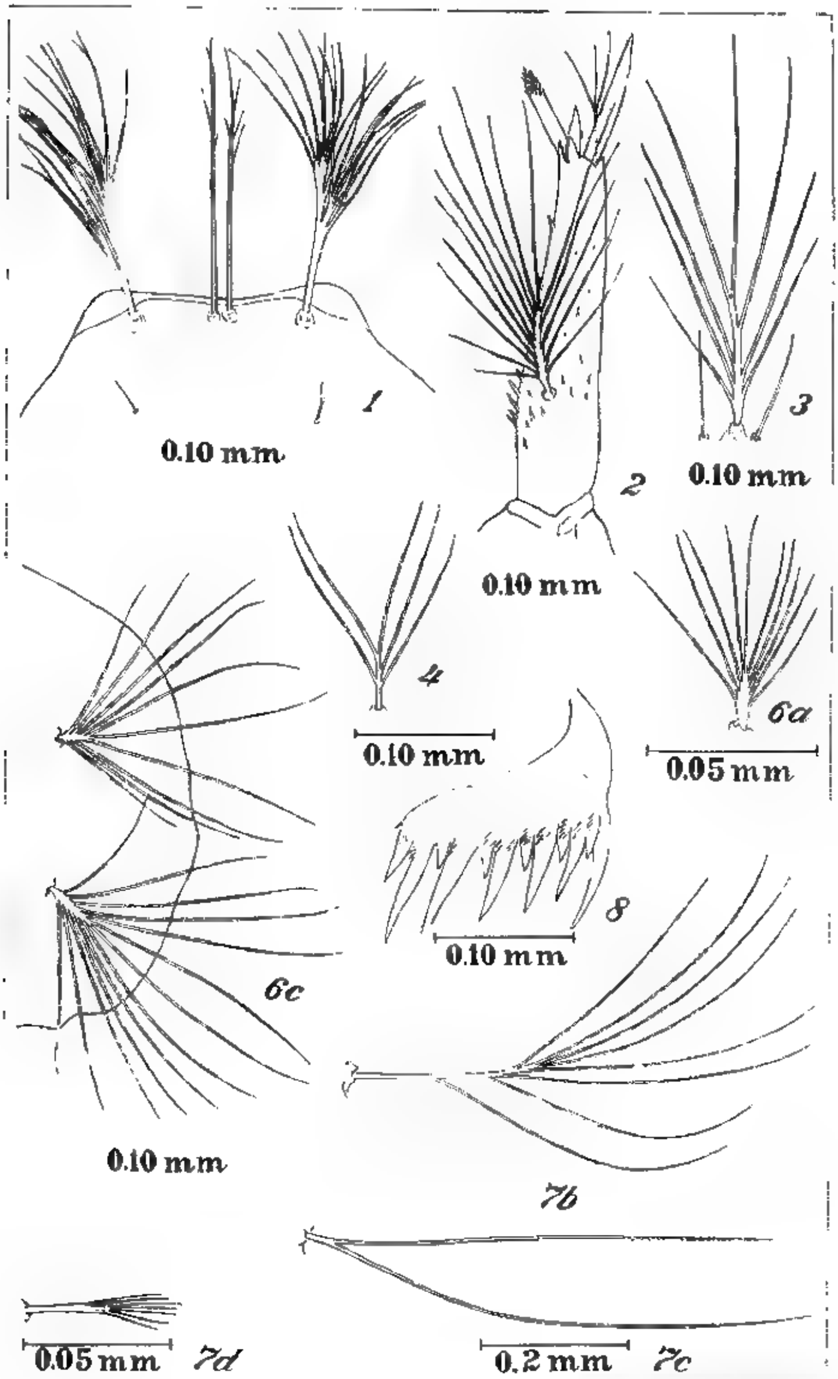

PLATE 3 ANOPHELES BAEZAI (?).

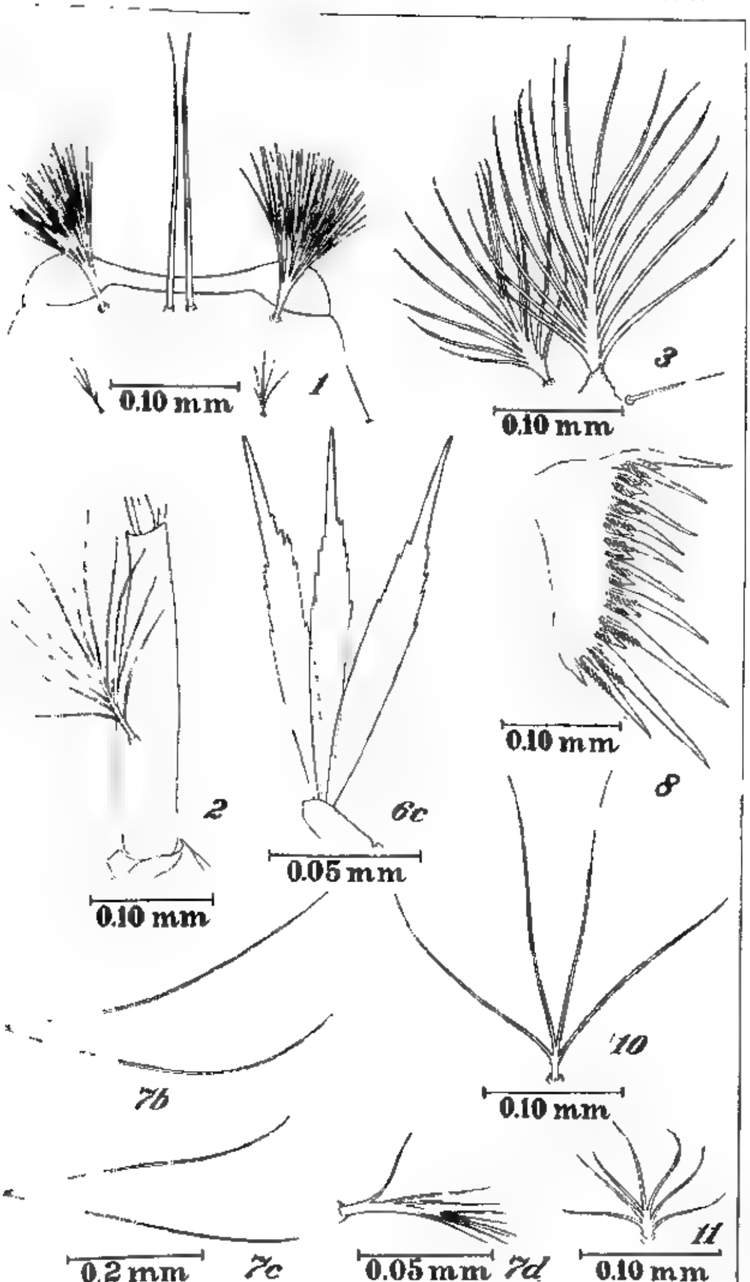

PLATE 4. ANOPHELES BARBIROSTRIS.

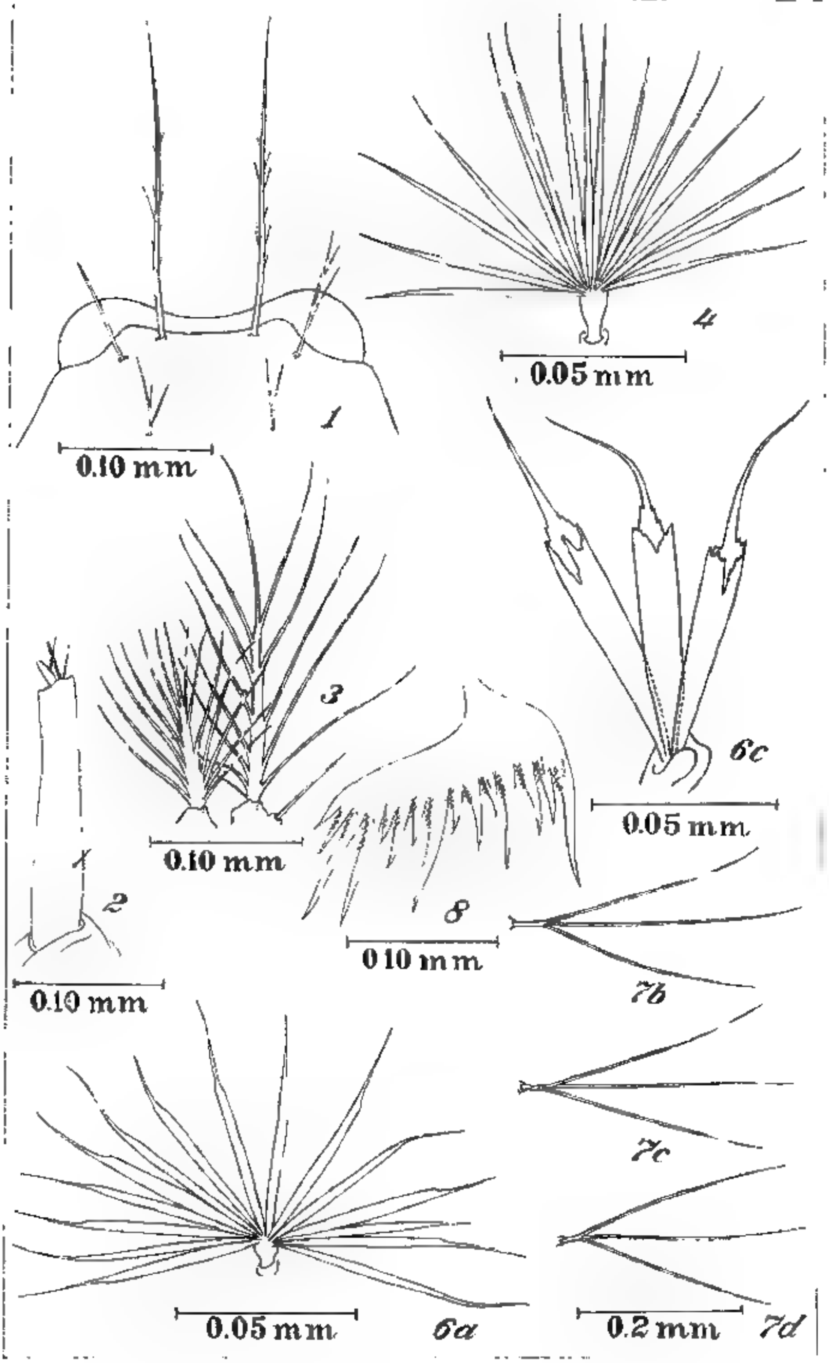

PLATE 5. ANOPHELES FILIPINÆ.

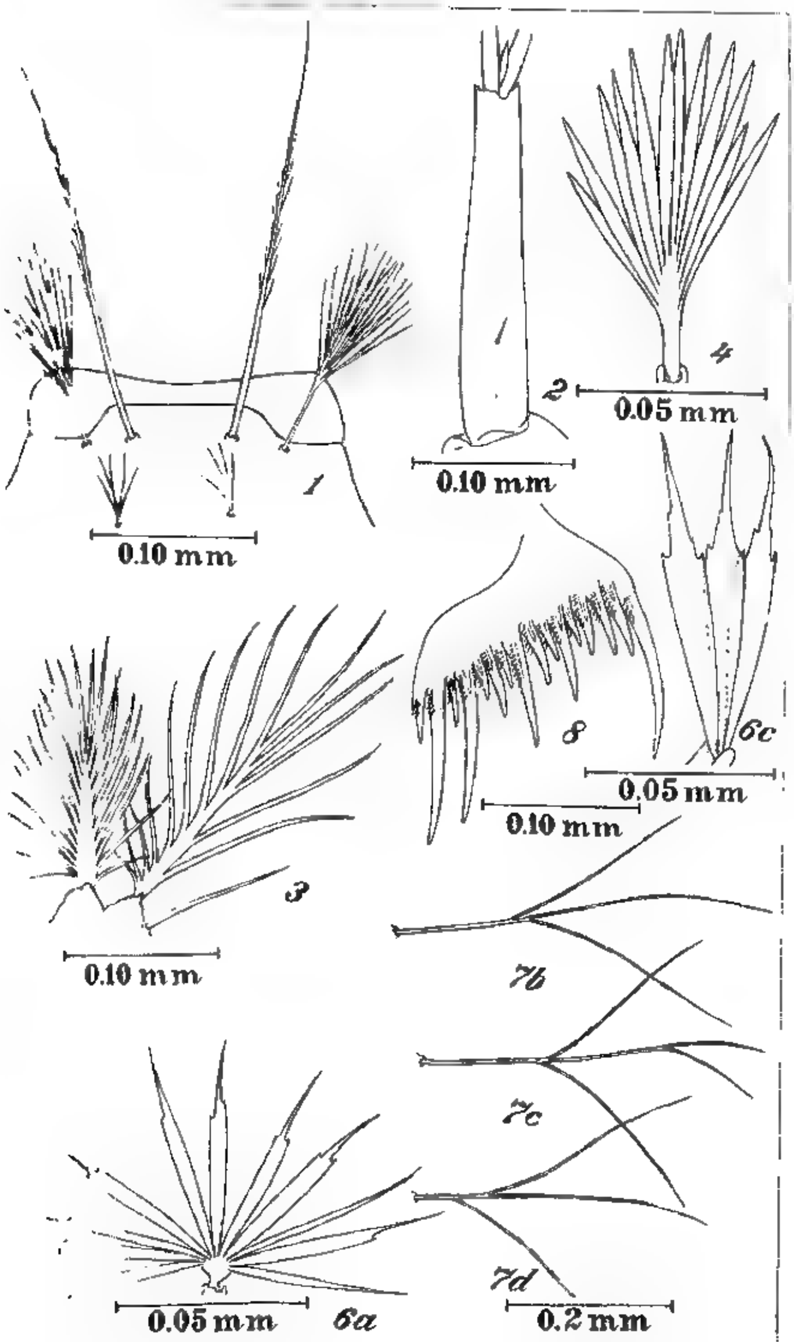

PLATE 6. ANOPHELES ANNULARIS.

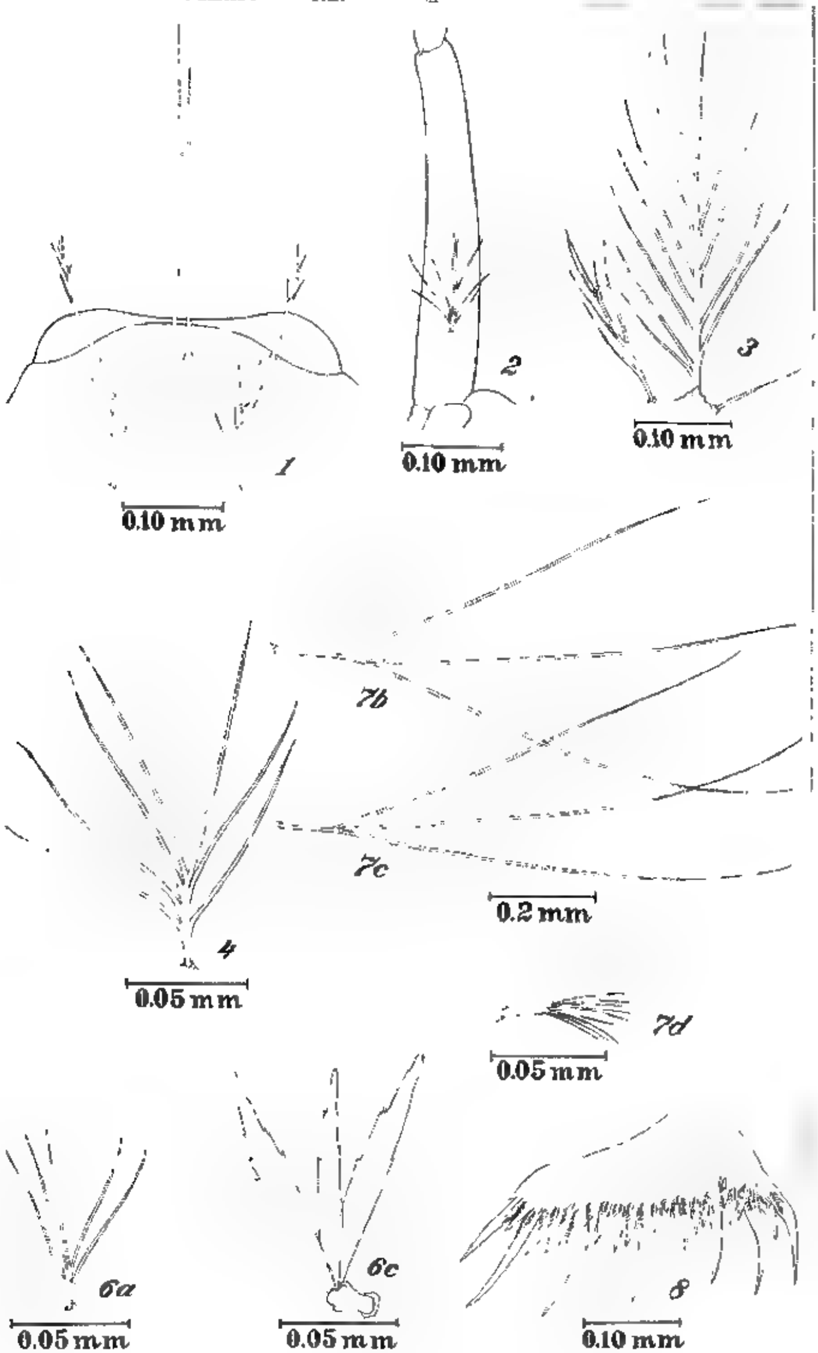

PLATE 7. ANOPHELES GIGAS VAR. FORMOSUS.

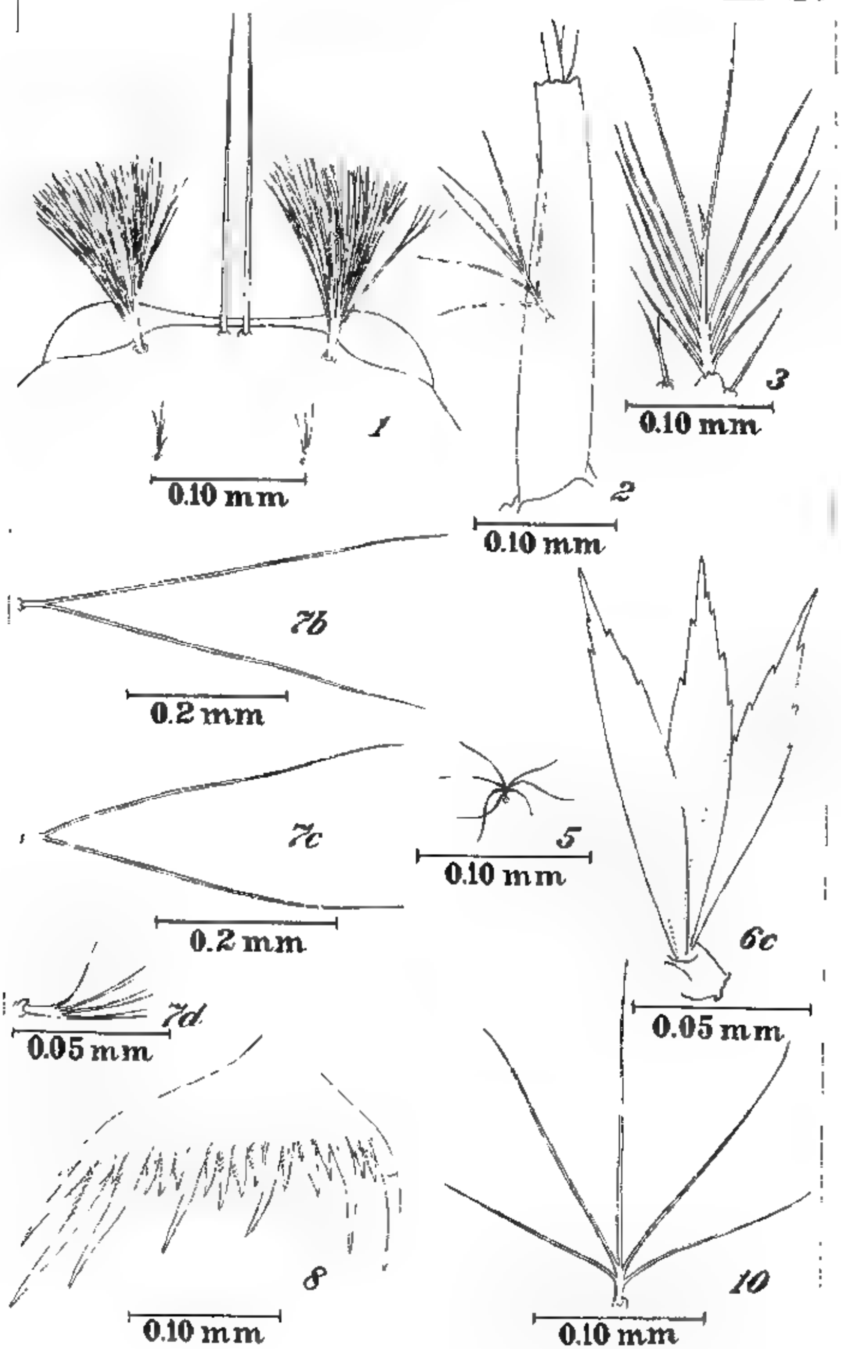

PLATE 8. ANOPHELES HYRCANUS VAR. NIGERRIMUS

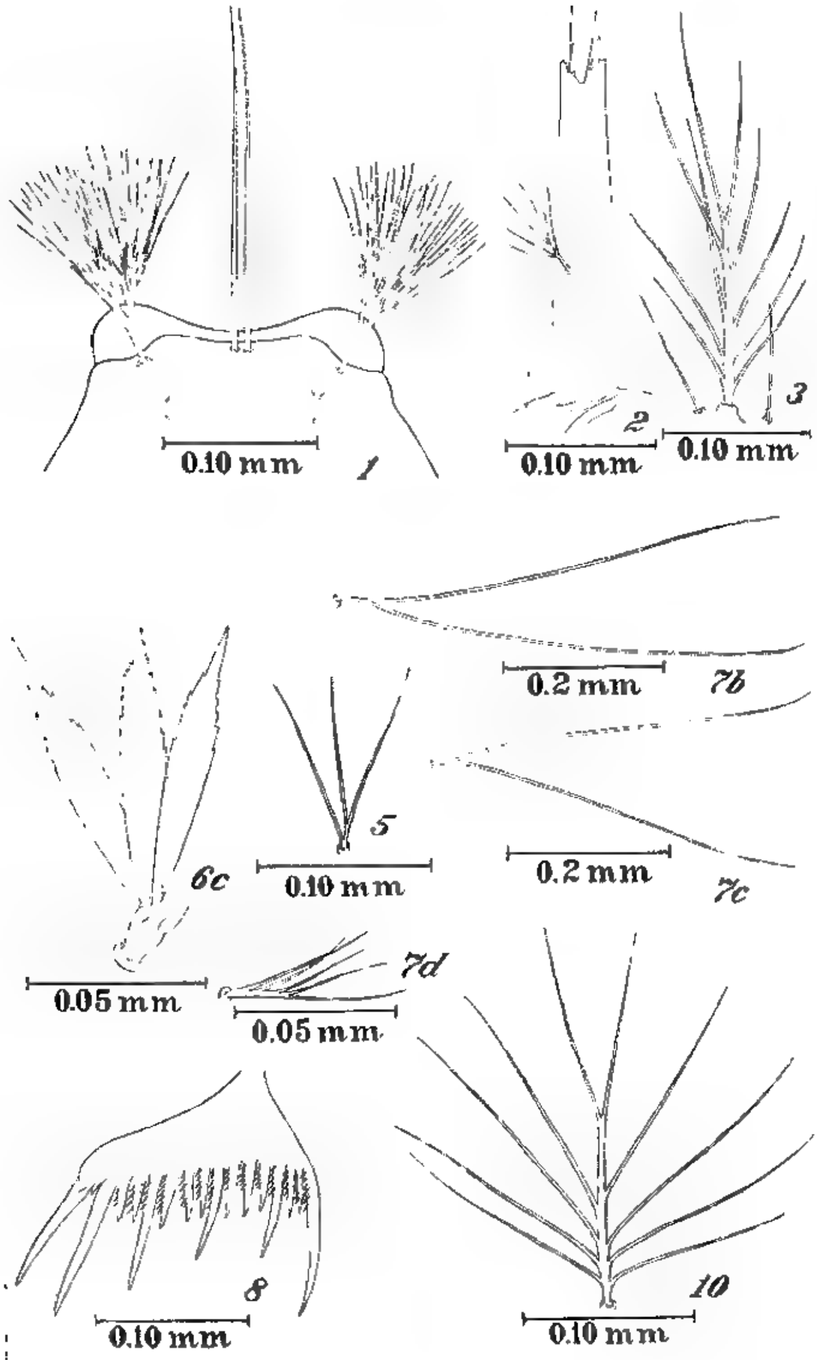

PLATE 9. ANOPHELES HYRCANUS VAR SINENSIS.

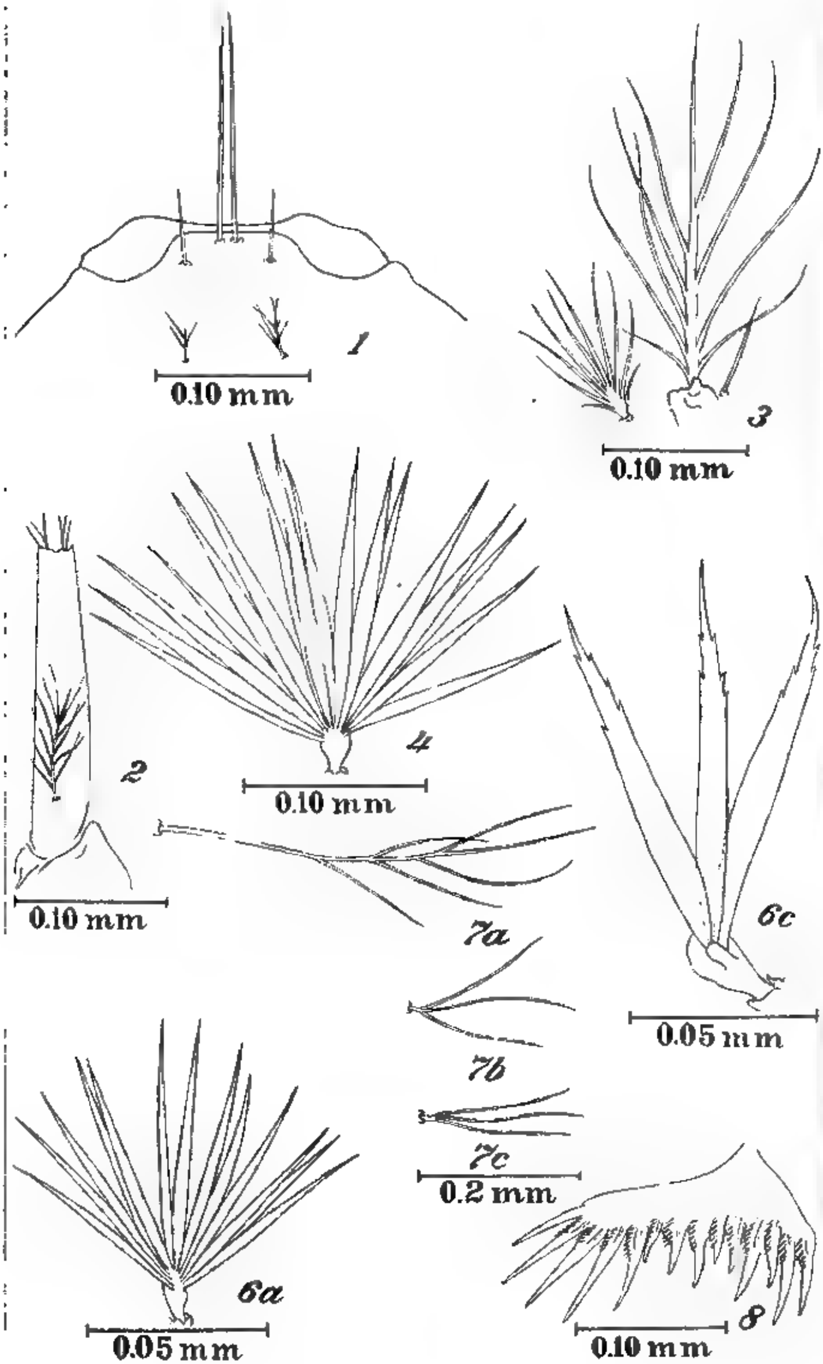

PLATE 10. ANOPHELES INSULÆFLORUM.

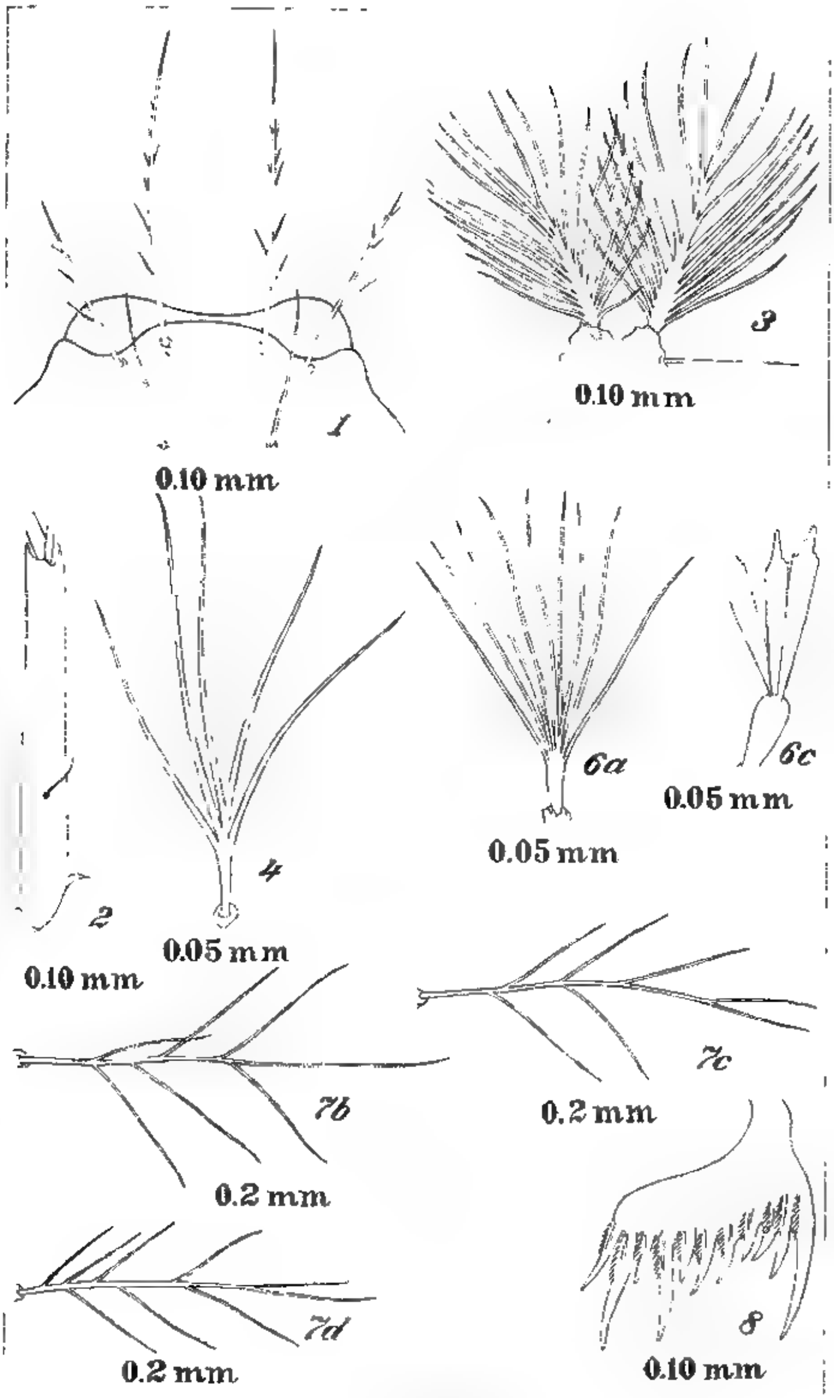

PLATE 11. ANOPHELES KARWARI.

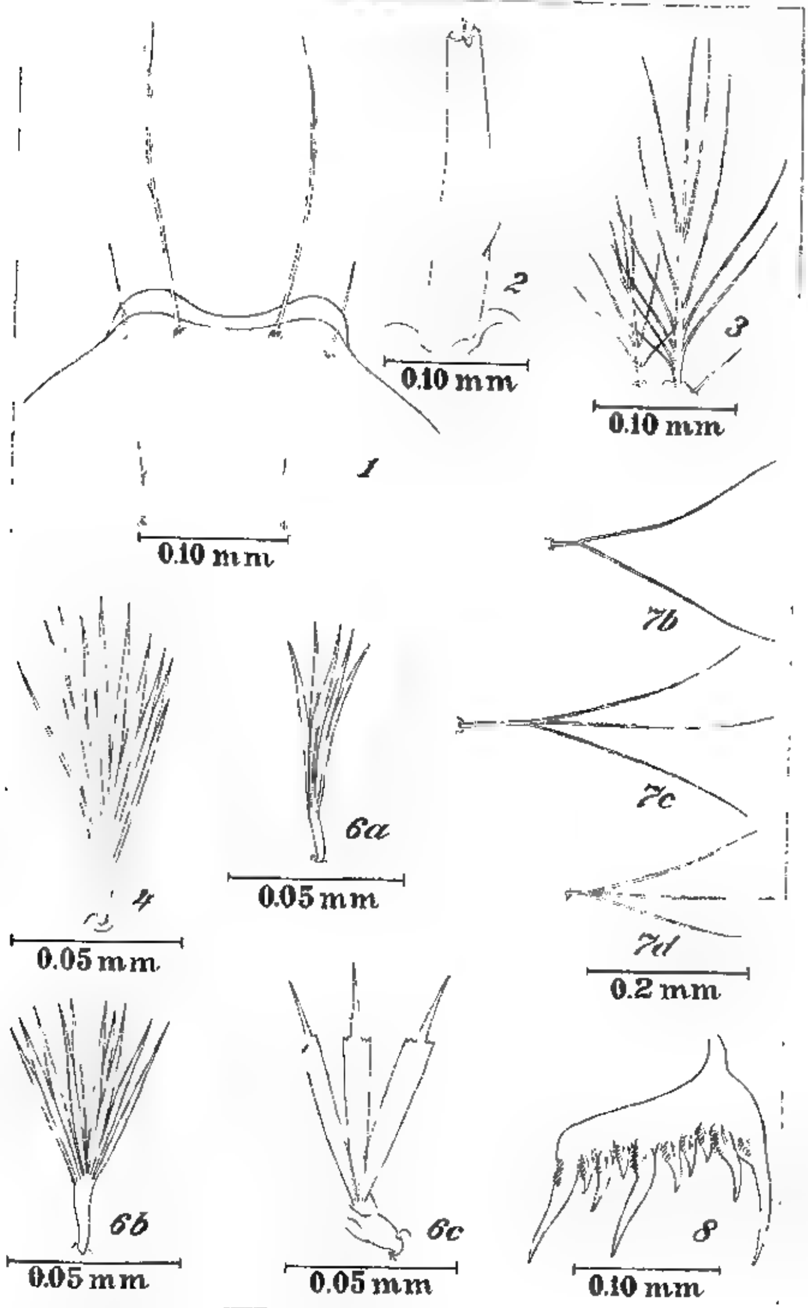

PLATE 12 ANOPHELES KOCHI

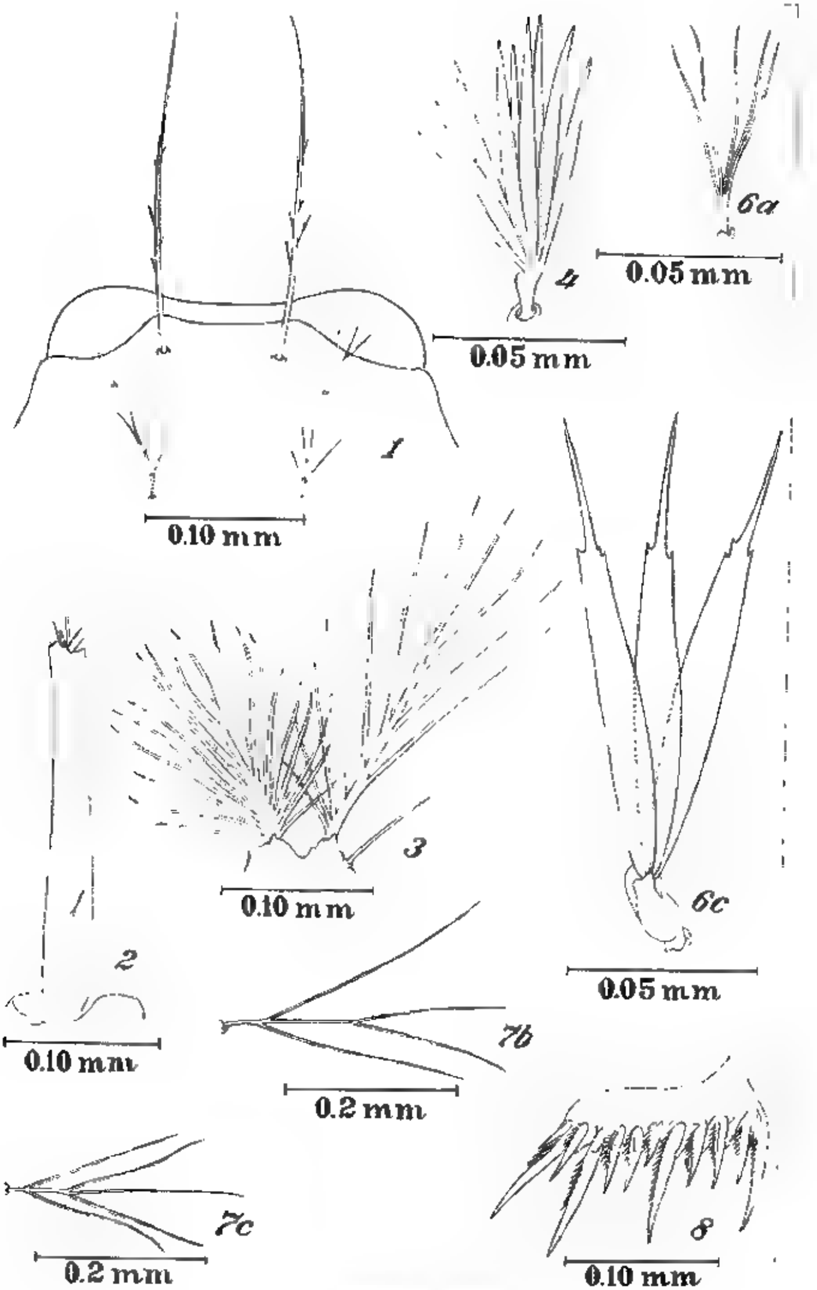

PLATE 13. ANOPHELES KOLAMBUGANENSIS.

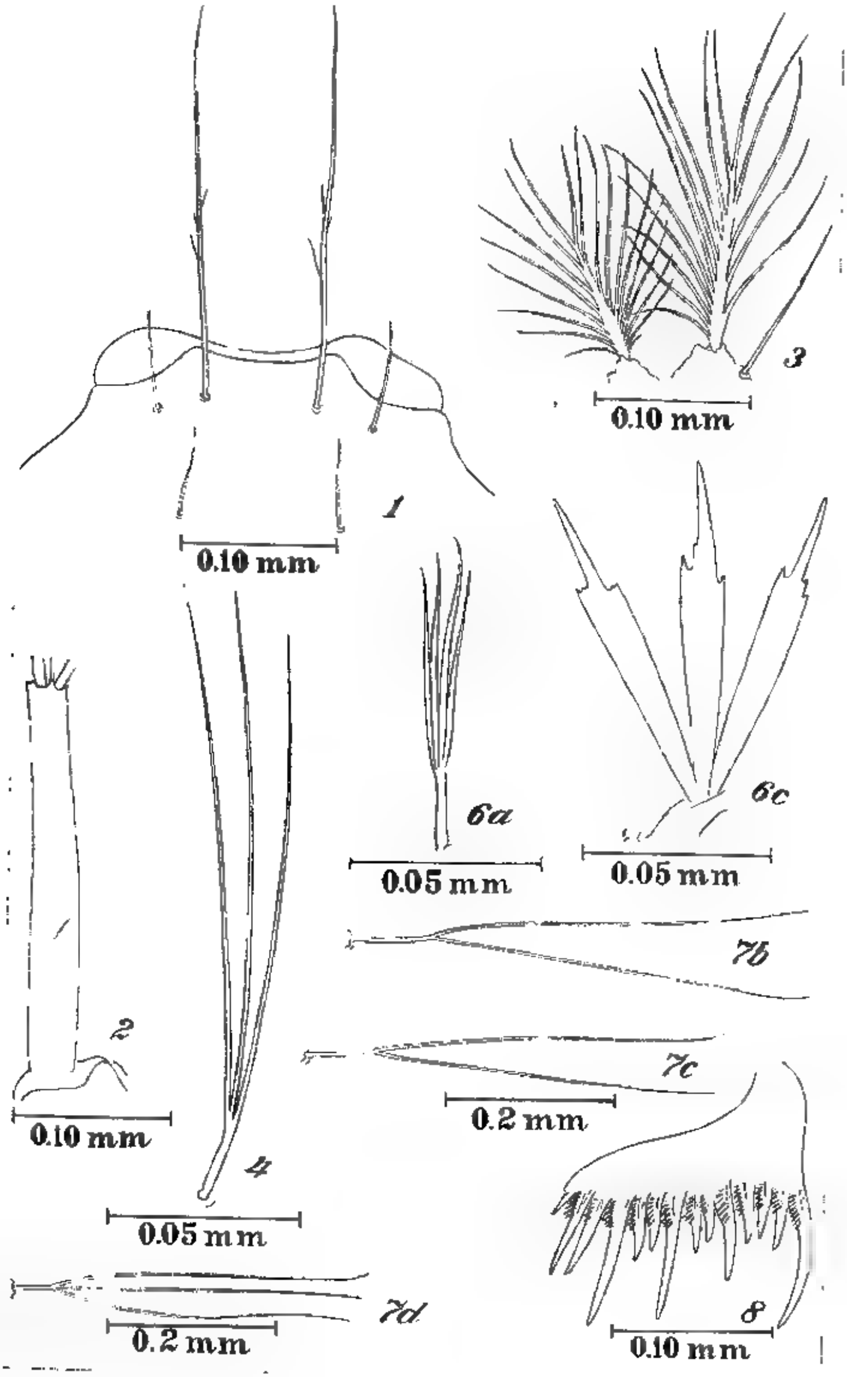

PLATE 14. ANOPHELES LEUCOSPHYRUS.

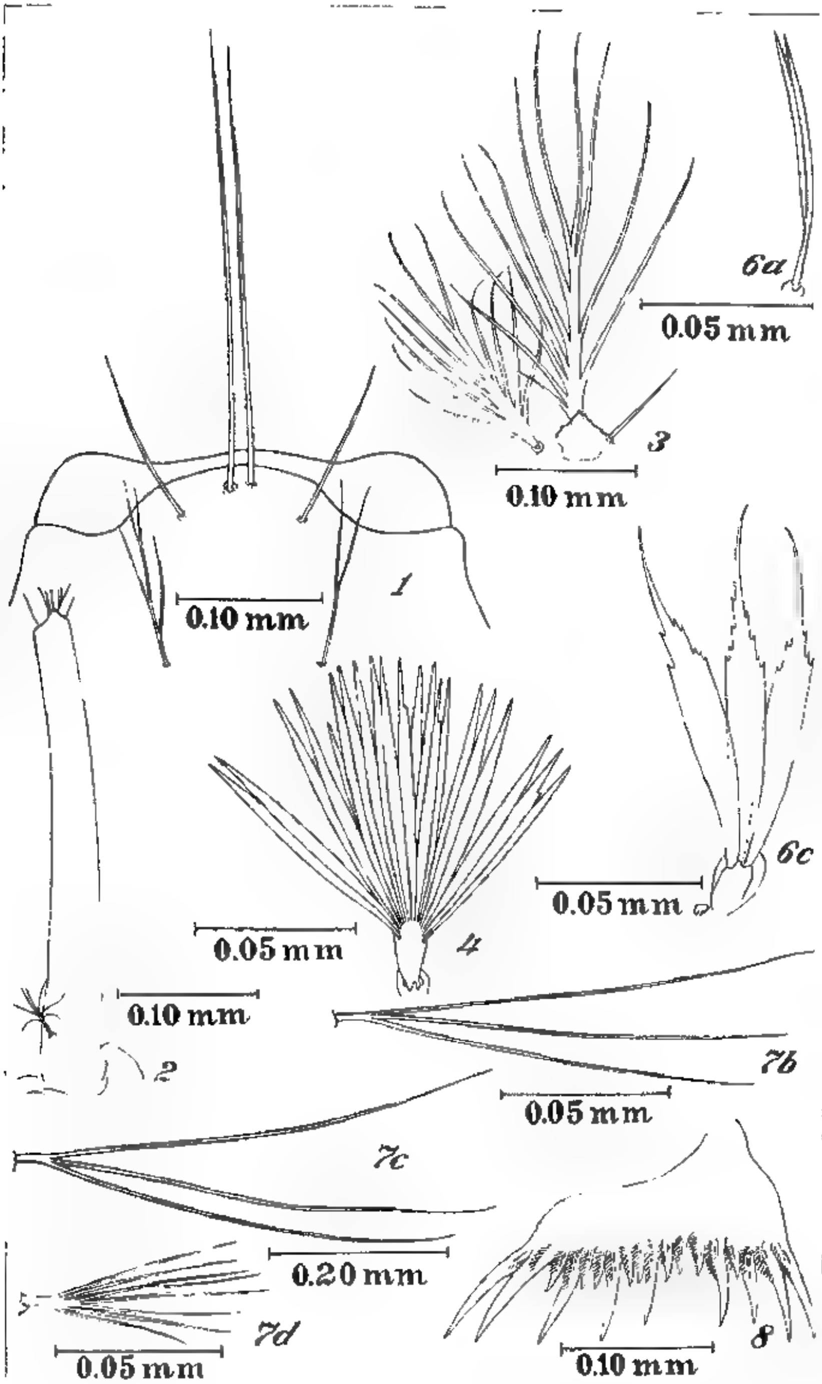

PLATE 15. ANOPHELES LINDESAYI VAR. BENGUETENSIS.

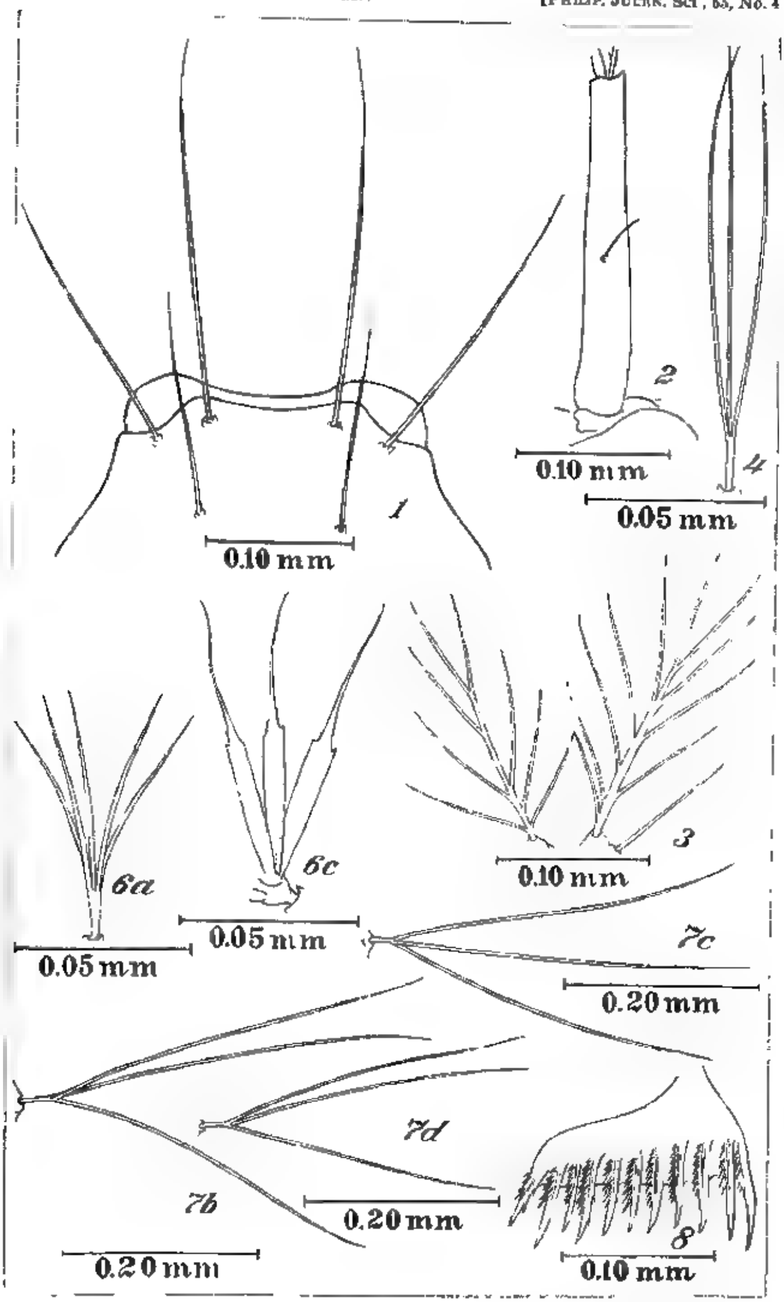

PLATE 16. ANOPHELES LITORALIS.

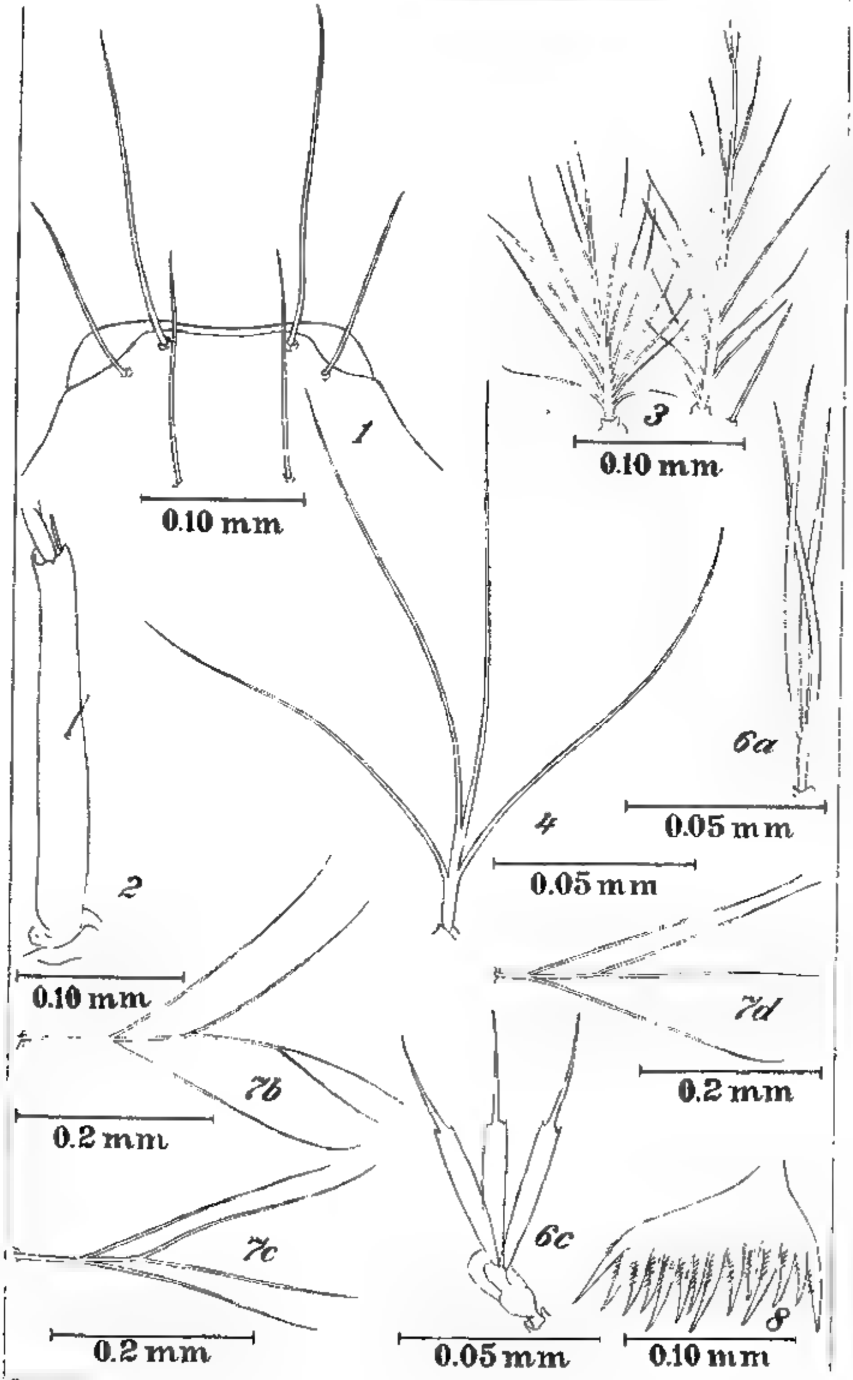

PLATE 17. ANOPHELES LUDLOWI.

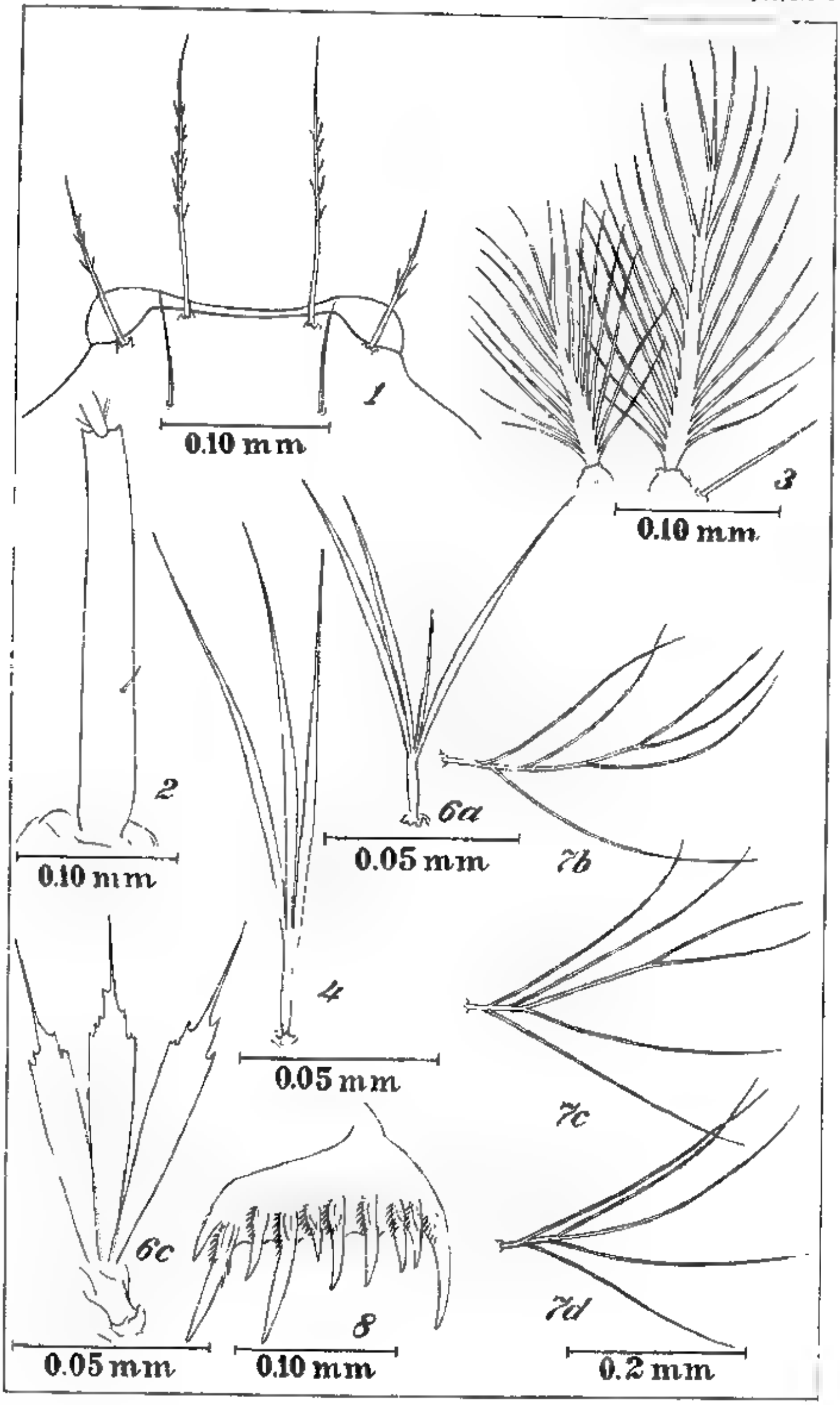

PLATE 18 ANOPHELES MACULATUS.

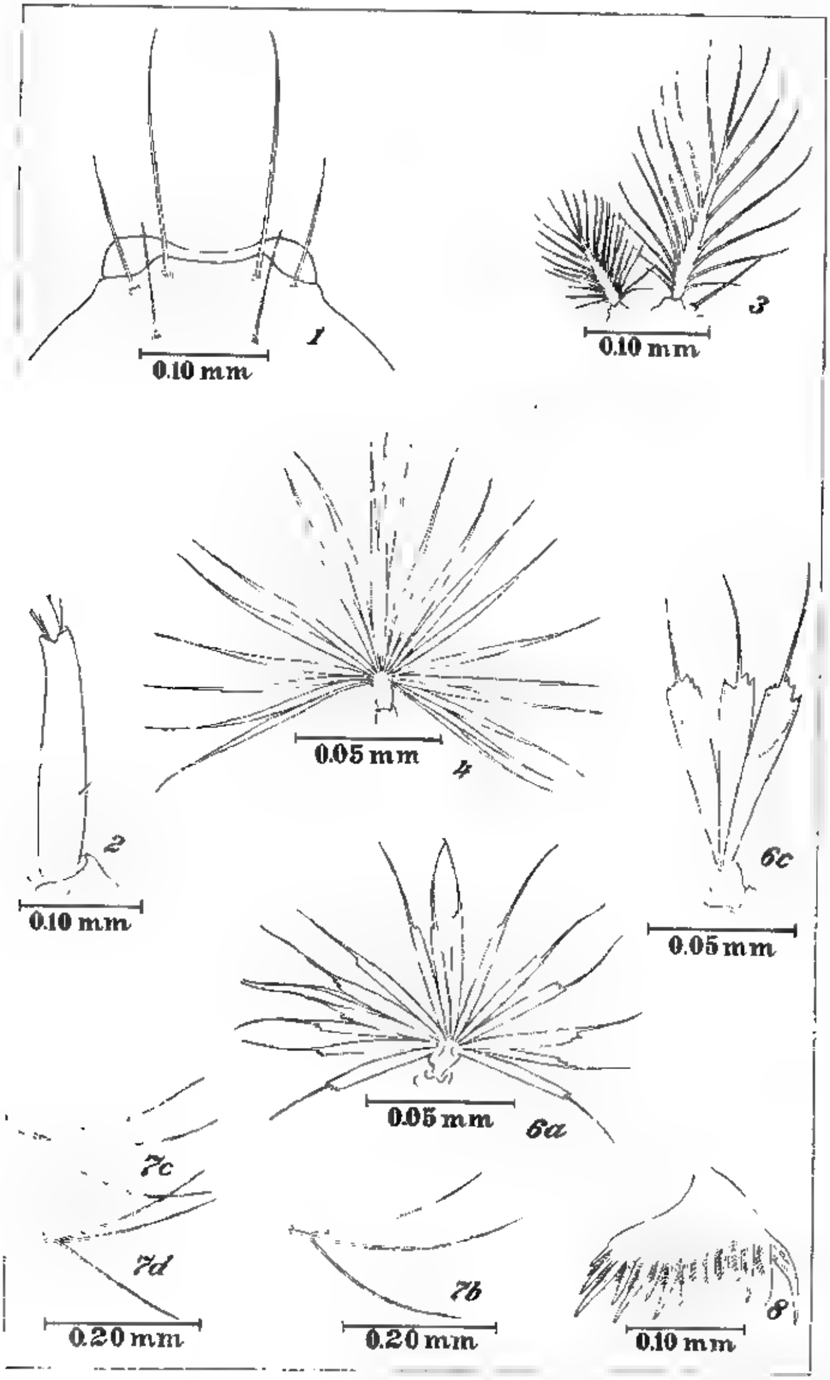

PLATE 19. ANOPHELES MANGYANUS.

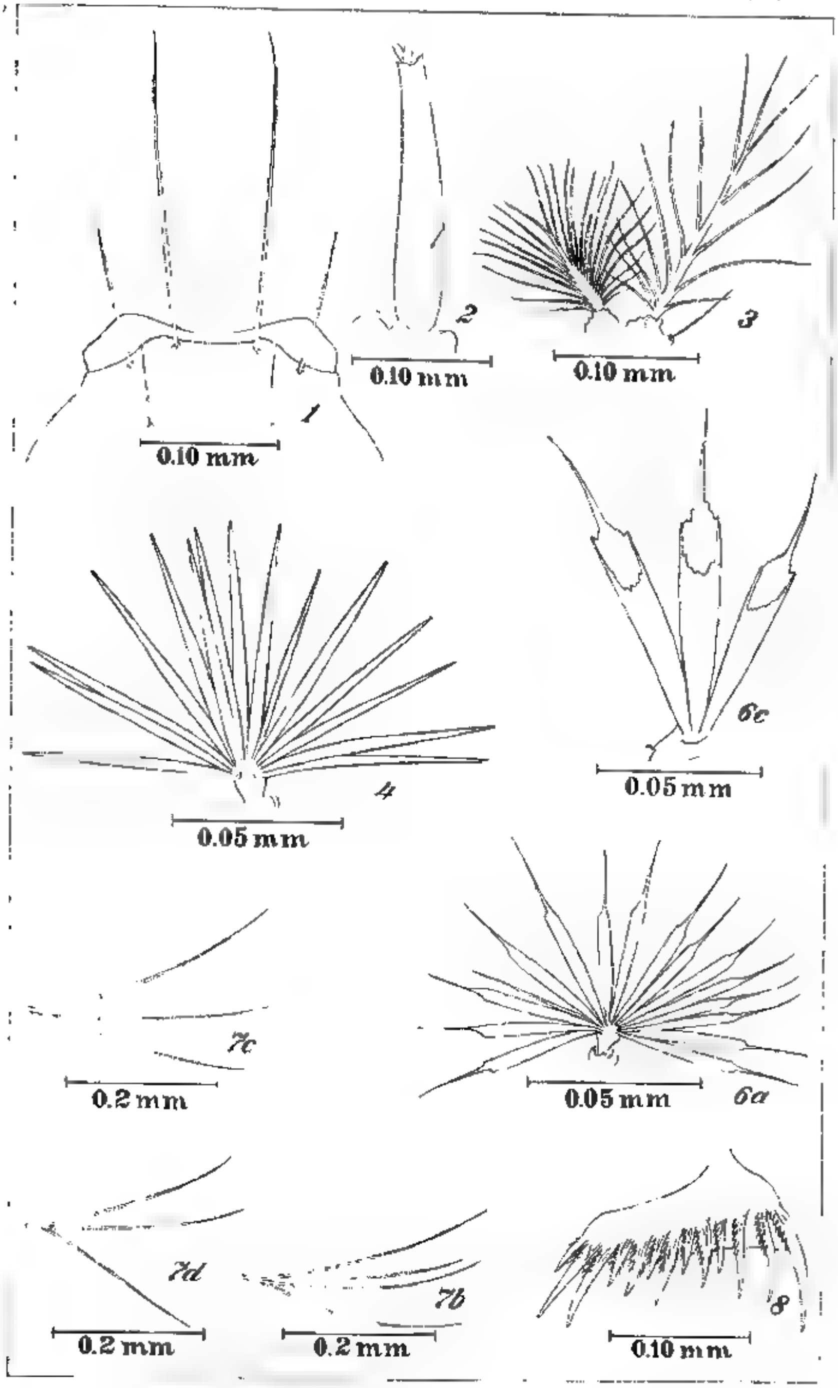

PLATE 20. ANOPHELES MINIMUS VAR. FLAVIROSTRIS

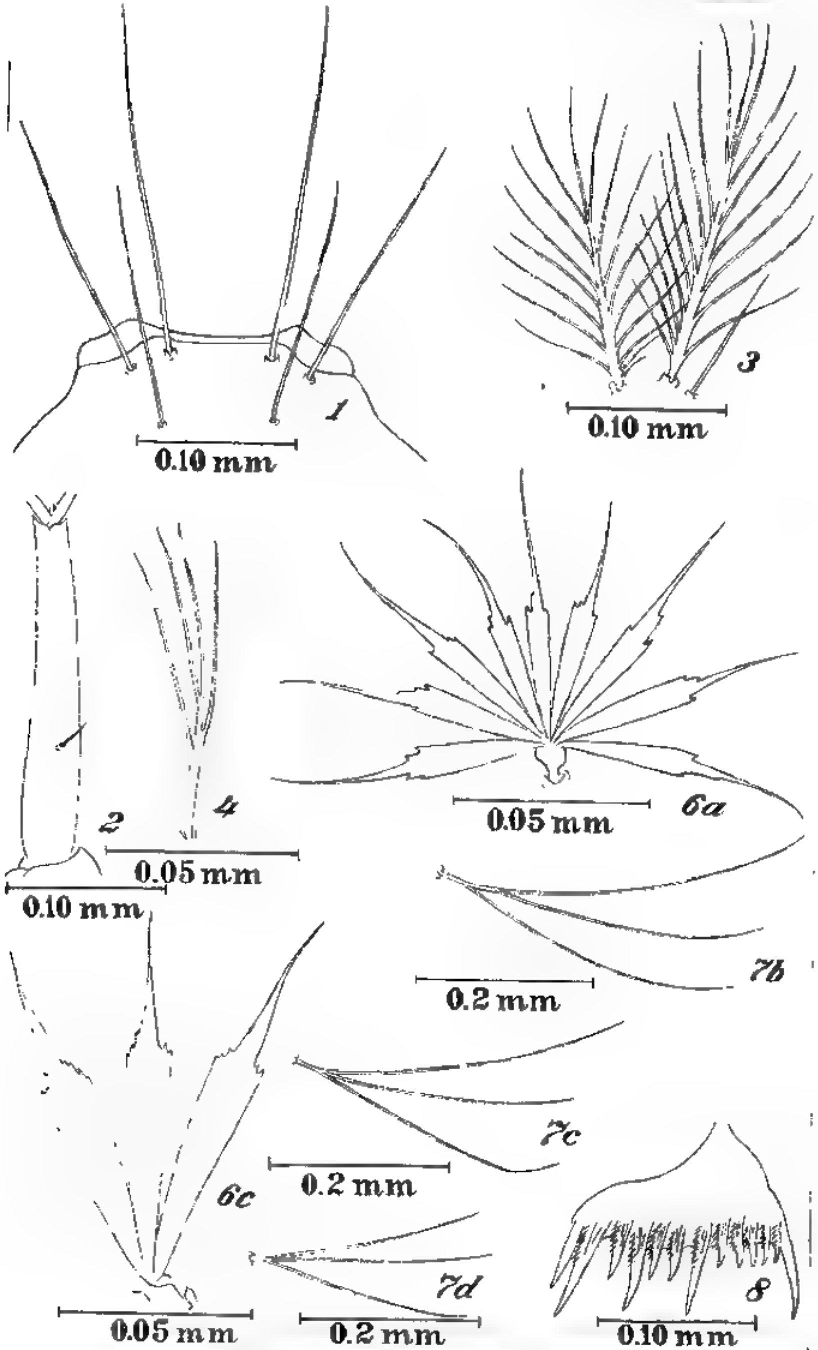

PLATE 21. ANOPHELES PARANGENSIS.

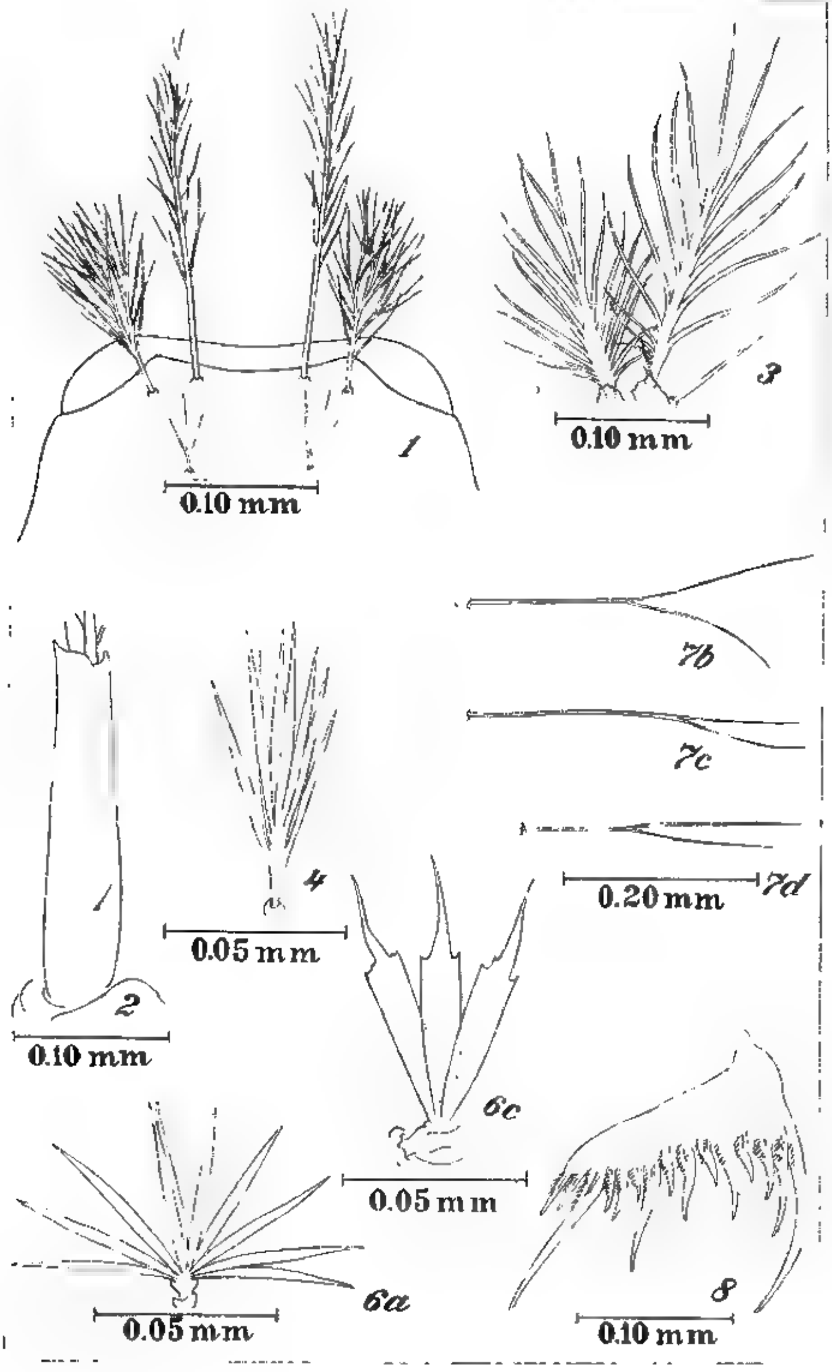

PLATE 22. ANOPHELES PHILIPPINENSIS.

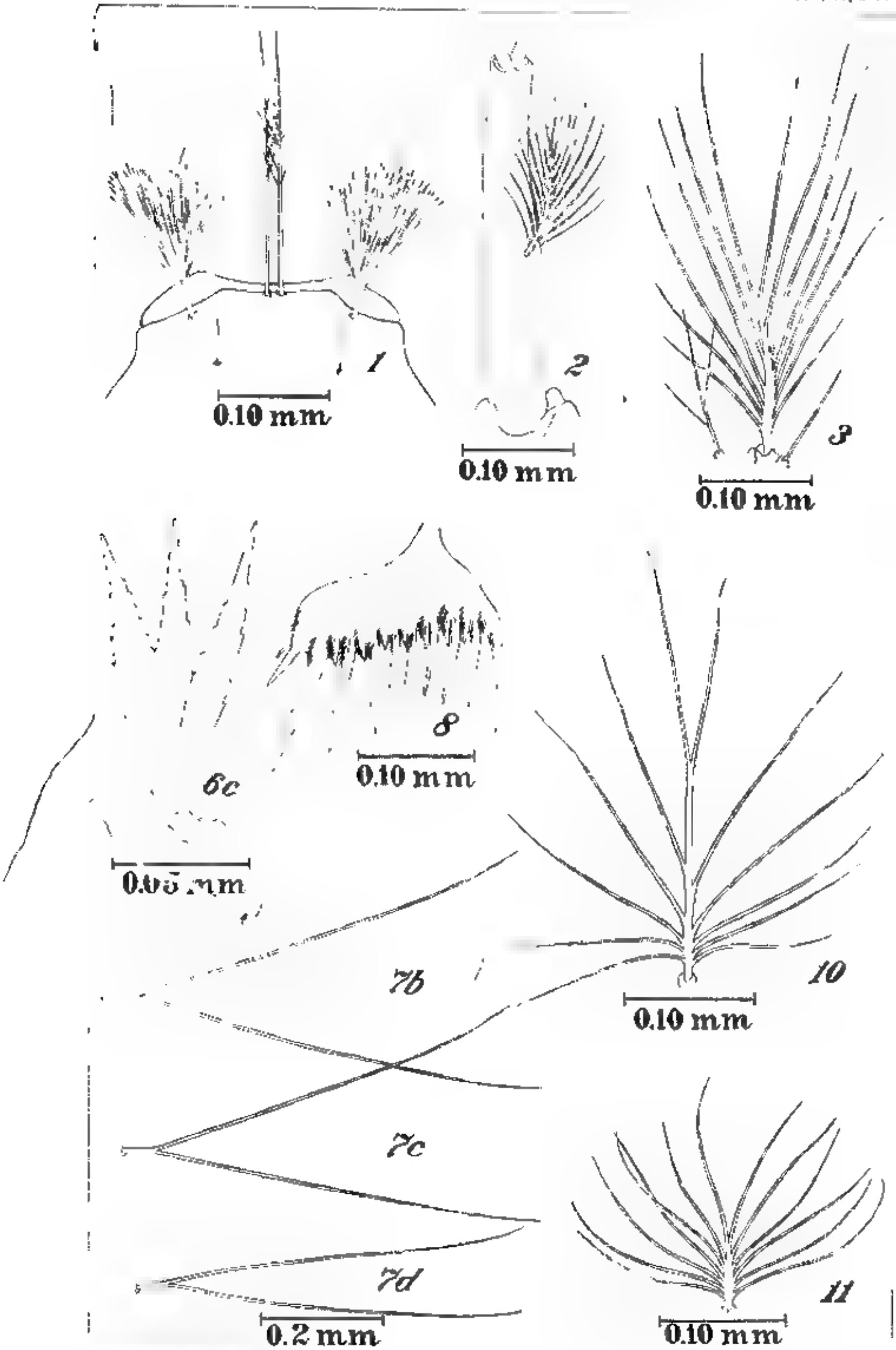

PLATE 23. ANOPHELES PSEUDOBARBIROSTRIS.

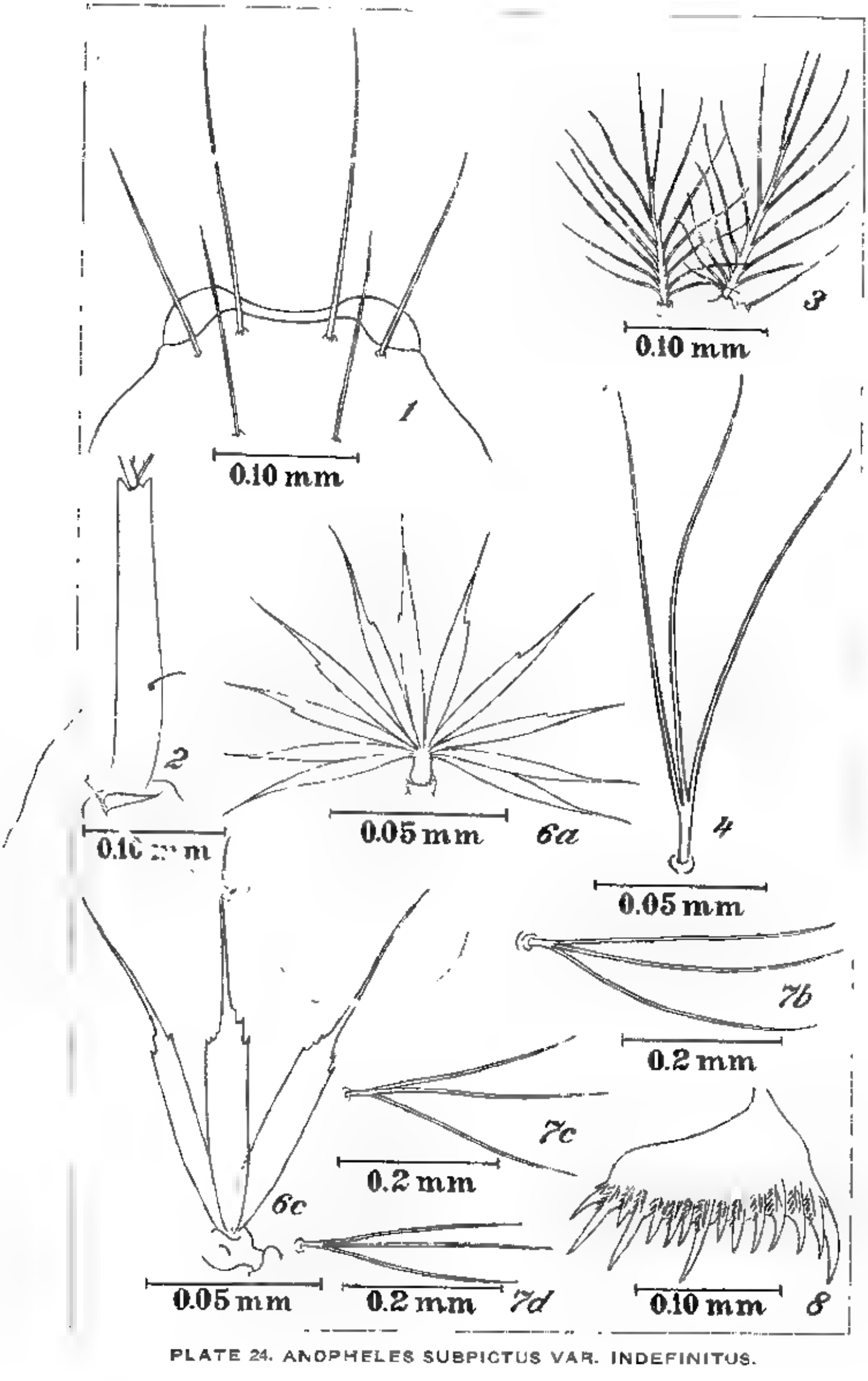

PLATE 24. ANOPHELES SUBPICTUS VAR. INDEFINITUS.

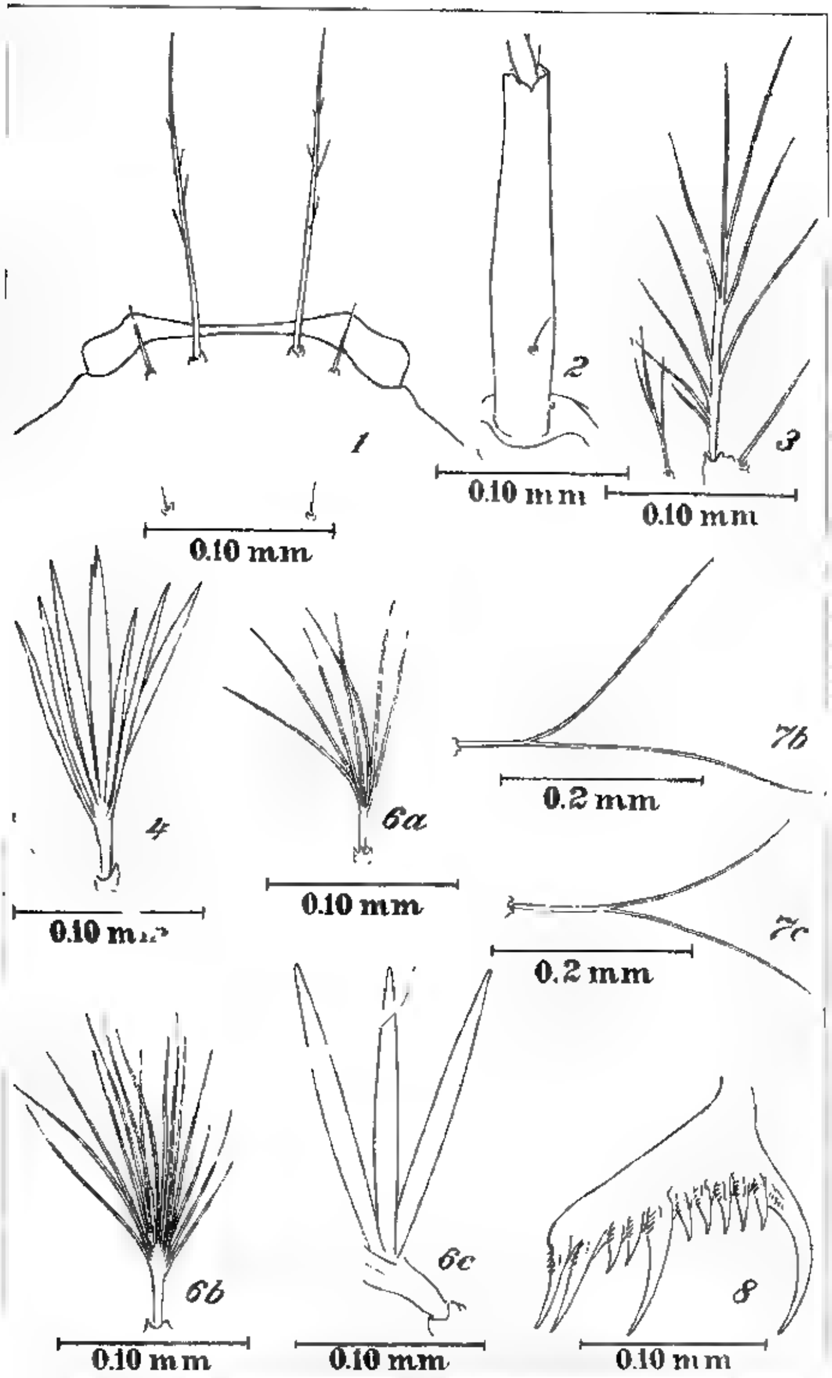

PLATE 25. ANOPHELES TESSELLATUS.

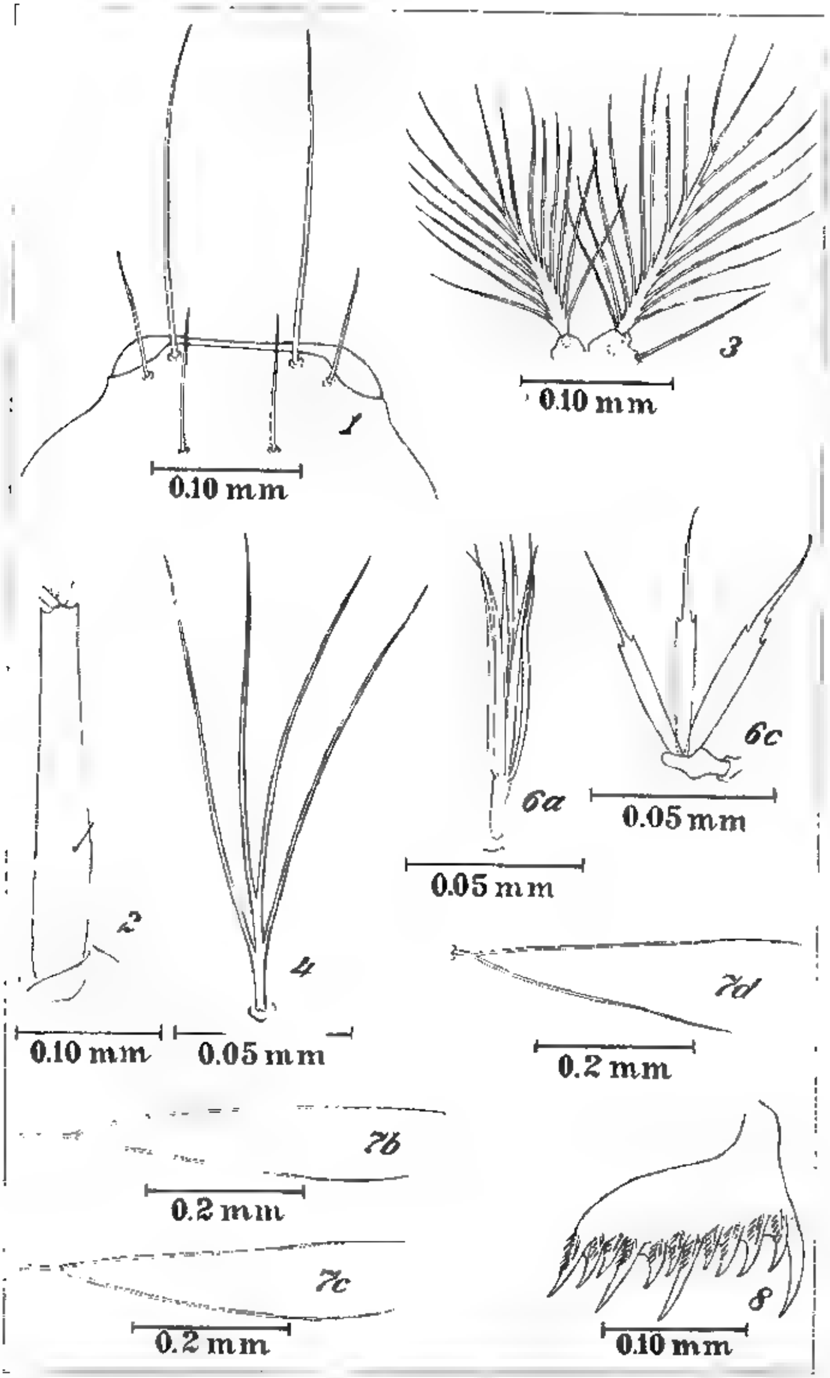

PLATE 26. ANOPHELES VAGUS VAR. LIMOSUS.

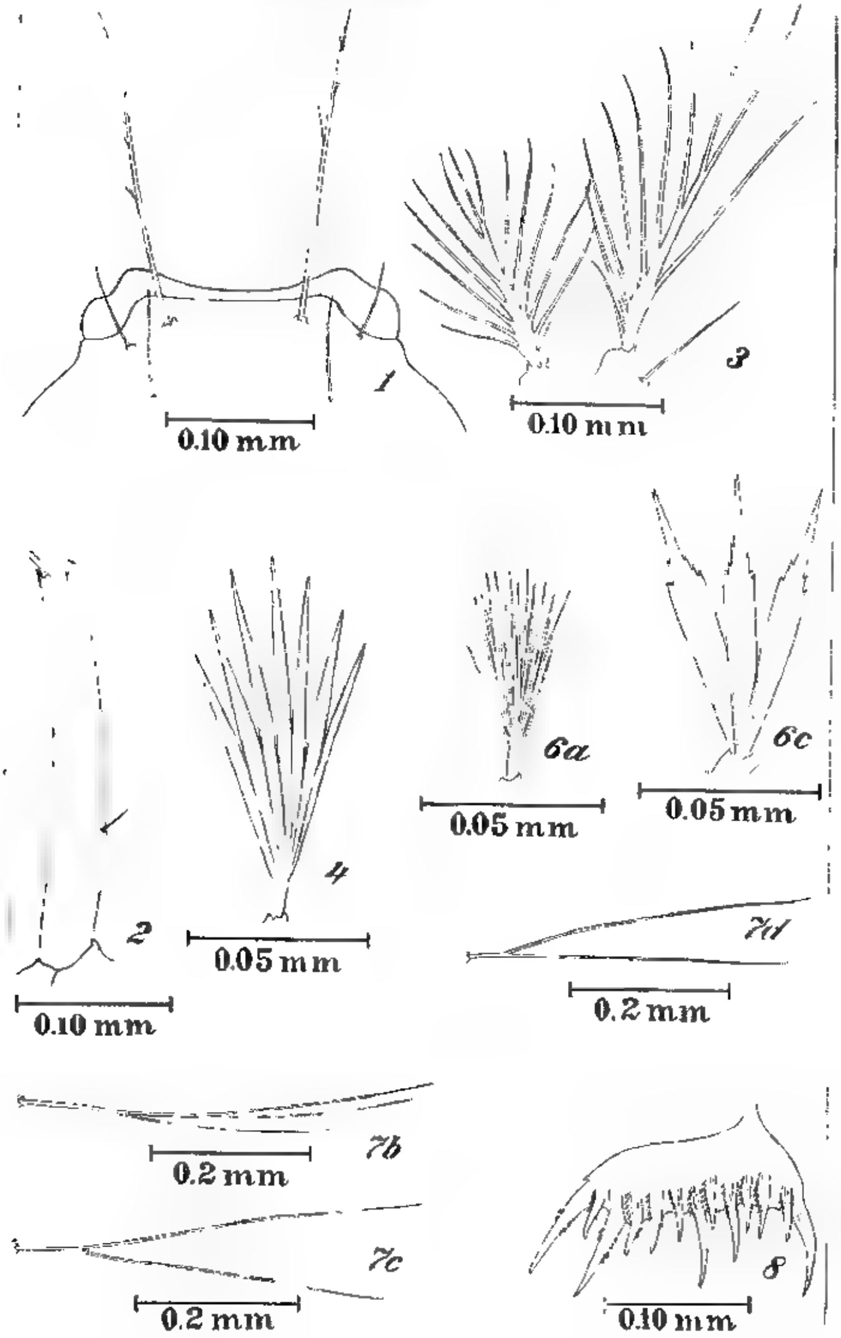

PLATE 27. BALABAC SPECIES (?).

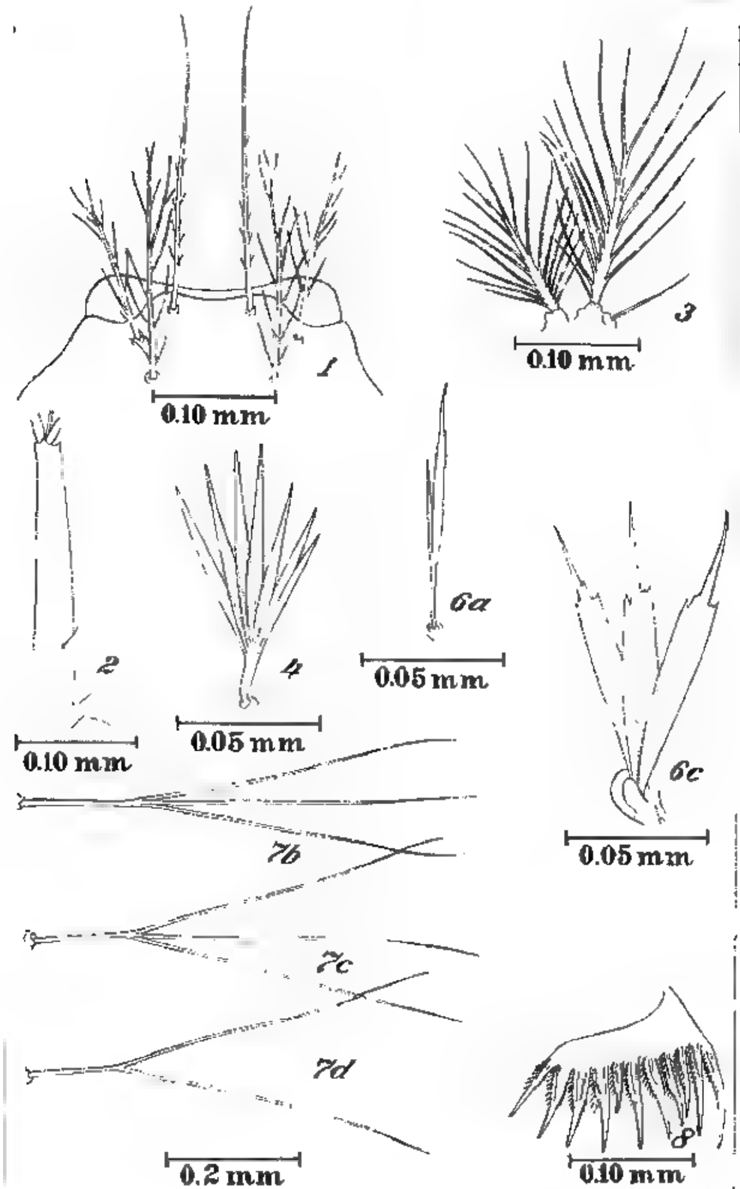

PLATE 28. ANOPHELES NEAR-LEUCOSPHYRUS (?).

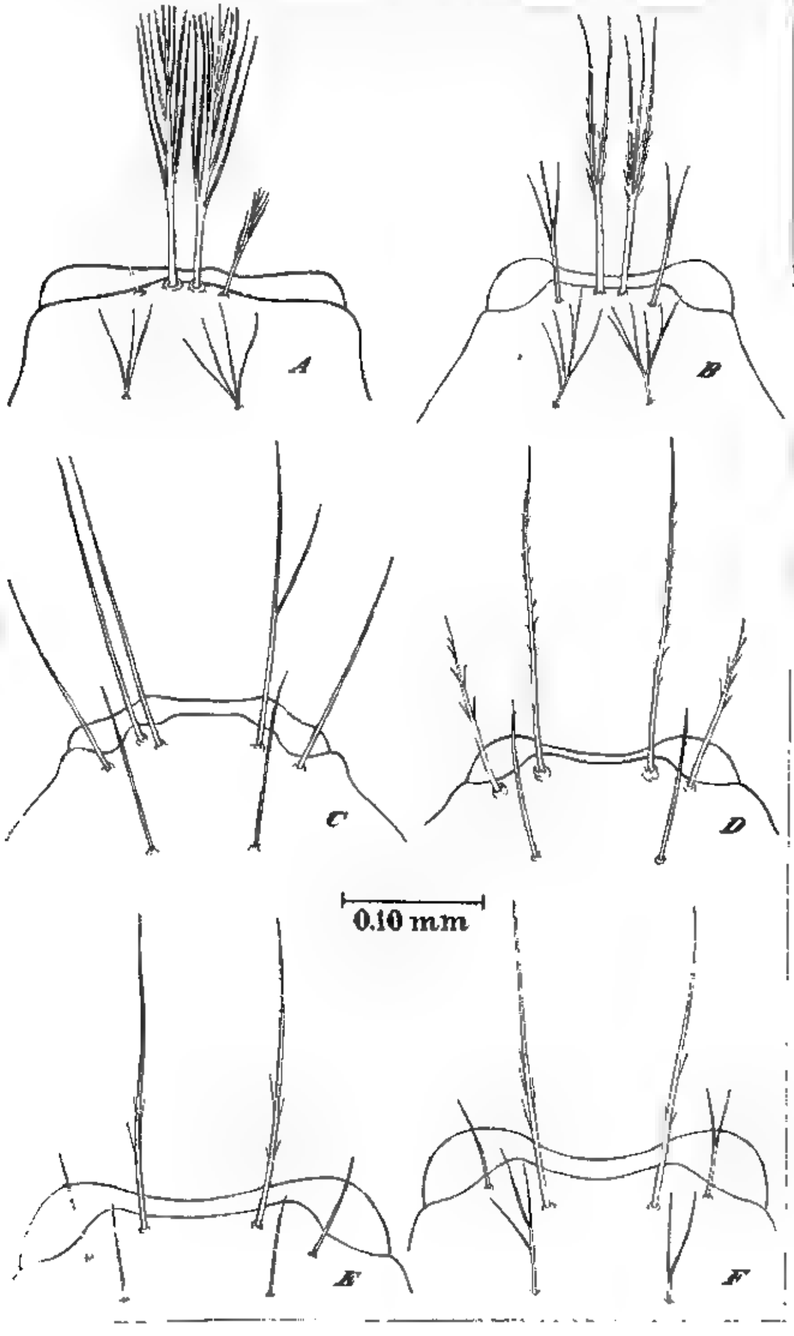

PLATE 29. VARIATIONS IN CLYPEAL HAIRS.

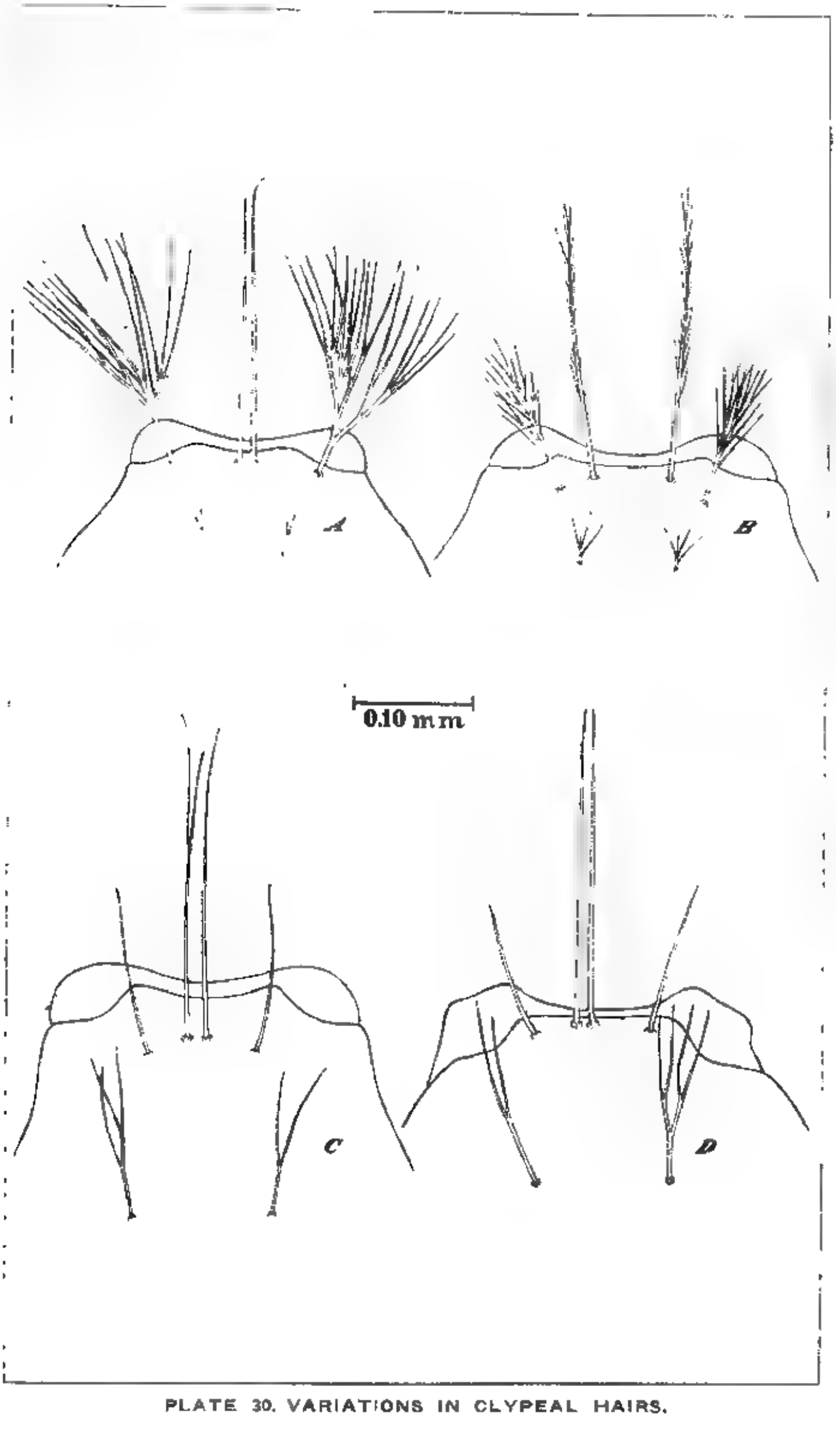

PLATE 30. VARIATIONS IN CLYPEAL HAIRS.

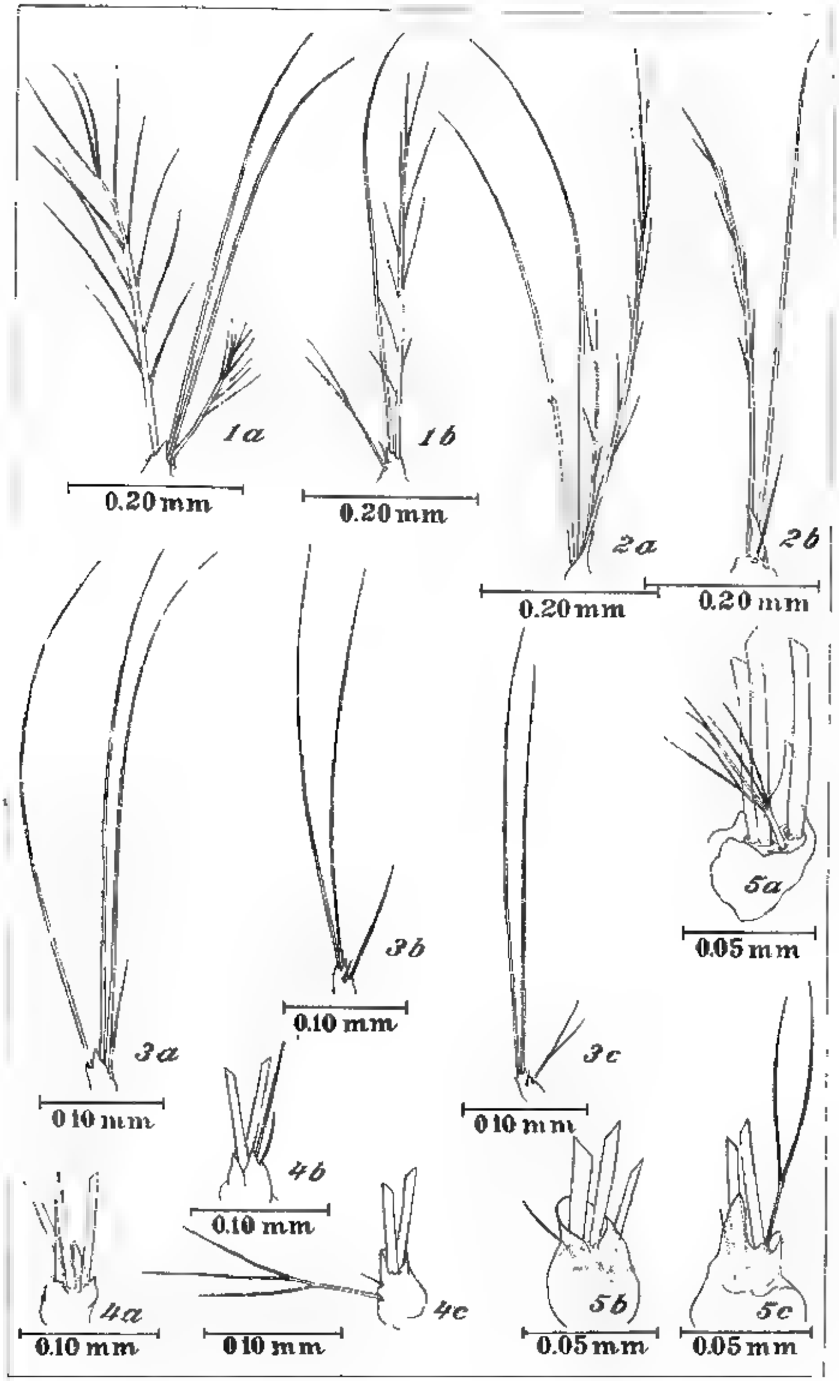

PLATE 31. PLEURAL HAIR GROUPS OF SOME UNCOMMON SPECIES.

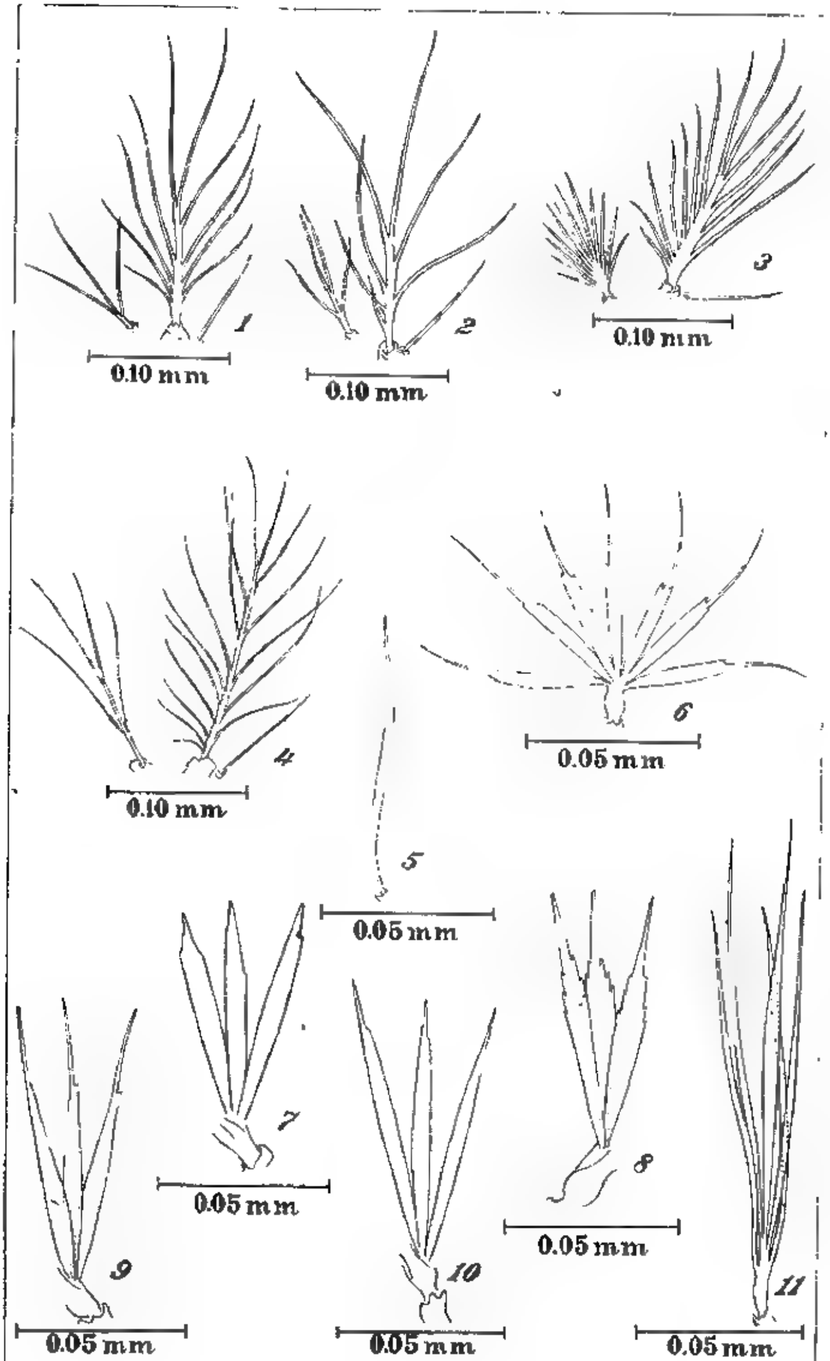

PLATE 32. VARIATIONS IN SHOULDER AND PALMATE HAIRS.

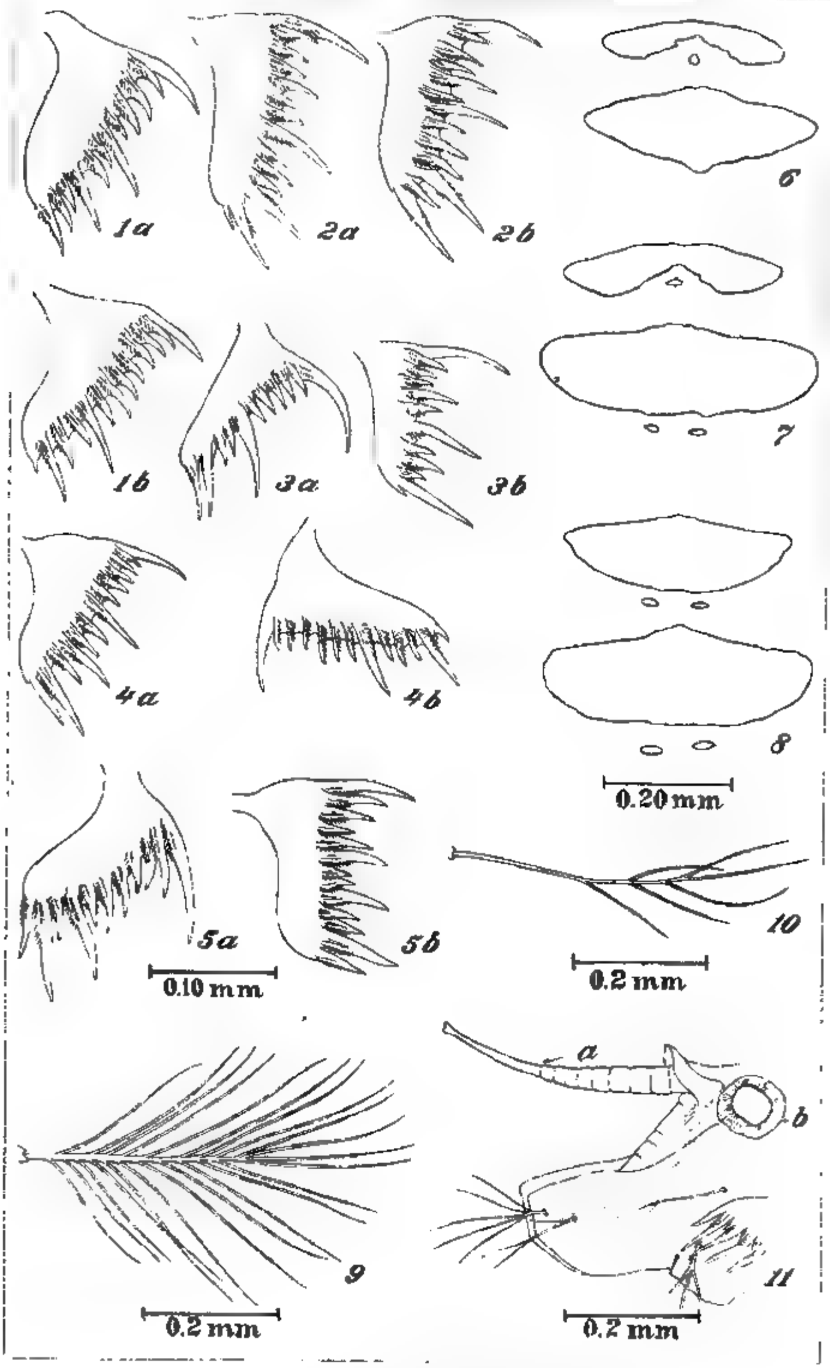

PLATE 33. VARIATIONS IN PECTENS, SOME LATERAL HAIRS III, TERGAL PLATES, AND A STIGMAL CLUB.

FRUITLET BLACK-ROT OF PINEAPPLE IN THE PHILIPPINES [1]

By F. B. SERRANO [2]

Of the Bureau of Science, Manila

SIX PLATES

INTRODUCTION

The fruitlet black-rot disease of pineapple, *Ananas comosus* (L.) Merr., was first noted by the writer as severely affecting fruits of the Smooth Cayenne variety and other unidentified hybrids in August, 1928, in Makar, Cotabato Province, Mindanao, where a few hectares of pineapple plantings were being cultivated by the Philippine Packing Corporation. Slicing hundreds of ripening fruits disclosed that 30 to 40 per cent were infected. Towards the end of the year and in the early part of 1929, the same infection was found on the Smooth Cayenne fruits in Santa Fe and Tangkulan, Bukidnon Province. Again in the summer months of 1931, 1932, and 1933, a study made on the Smooth Cayenne fruits coming from Cavite, Bulacan, Laguna, Nueva Ecija, and Pangasinan Provinces, in Luzon, resulted in the identification of the same trouble. The origin of the disease is not known. It is quite probable, however, that it was imported with Smooth Cayenne planting materials from Hawaii, where the disease is known to be present though not severe. The fact that it is not found to affect any of the native varieties seems to justify this view.

HISTORY AND GEOGRAPHIC DISTRIBUTION

As early as 1898, Tryon (20) studied in Queensland a disease of pineapple which he called fruitlet core-rot, affecting the Smooth

[1] The second part of this article dealing with control measures will soon be published in this journal.

[2] The writer wishes to express his gratitude to the Philippine Packing Corporation for its valuable help in making this investigation possible. Thanks are also due to Dr. E. Quisumbing, of the National Museum Division, for reading the manuscript and for the encouragement he has given me in its preparation.

Cayenne and Prickly Queen varieties. *Penicillium* and *Monilia* have been found in the spots and were thought to have gained entrance into the ovarian cavities of the fruitlet through wounds made by the pineapple mite (*Tarsonemus ananas* Tryon), in the flower bowl. Lucas(10) reported a somewhat similar disease of the Ripley and Queen varieties of pineapple in Jamaica, in 1907. Nowel(12) stated in his report of 1923 that Howard studied a black eye-spot disease of West Indian pineapples, in 1901, and found *Penicillium* associated with the spots. Pole-Evans(15) reported in 1924 a brown-spot malady of pineapple in South Africa which he found to be associated with the same fungus.

Whether or not the maladies mentioned above are identical with the Philippine fruitlet black-rot disease cannot possibly be ascertained owing to the incomplete descriptions of their symptoms and the inadequate study of their causal organisms. Among the available literature on the subject the only report which gives a description that tallies in almost every detail with the Philippine fruitlet black-rot is that of Barker,(1) from Cap Haitien, Haiti, in which he stated that he had isolated from the earliest stages of the disease a pale yellowish bacterium with rugose colonies, with which he was able to reproduce the typical black-rot disease through artificial infection.

ECONOMIC IMPORTANCE

The result of examinations made of 6,936 fruits of the Smooth Cayenne variety in Cotabato and Bukidnon Provinces, Mindanao Island, from 1928 to 1930, showed that the infection by the fruitlet black-rot disease oscillated approximately from 27 to 47 per cent, of which about 12 per cent were unfit for canning. Such an infection may not mean much loss to the dealers of fresh fruits, but it certainly gives a lot of inconvenience and trouble to the canners, in addition to the material loss which it entails. Table 1 shows that only 66.20 per cent of the fruits examined are healthy and free from any discoloration; 21.40 per cent are slightly affected, while the rest are so badly diseased that they are absolutely unfit for human consumption. In view of this seriousness and in view of the bright future of the pineapple industry in this country which may be jeopardized by the presence of this malady, it was deemed desirable to study the problem with the purpose of devising means of control.

TABLE 1.—*Showing percentages of healthy and infected fruits among 6,936 Smooth Cayenne fruits.*

Year.	Place.	Number of fruits.	Observations.			
			Healthy.	Slightly Infected.	Severely infected.	Total loss.
1928	Makar, Cotabato	128	63.8	24.4	10.1	1.7
1929	Tangkulan, Bukidnon	1,440	67.3	29.6	2.8	0.3
1929	do	2,814	73.2	24.8	1.3	0.7
1930	Santa Fe, Bukidnon	352	62 2	23.1	11.1	3.6
1930	do	220	73.1	18.5	6.7	1.7
1930	do	807	72.4	20.3	5.9	1.4
1930	do	352	62.2	23.1	11.1	3.6
1930	do	369	64.0	17.0	15.3	3.7
1930	do	238	55.3	18.2	16 7	9.8
1930	do	216	68.6	15.0	12.6	3.6
	Average per cent		66.20	21.40	9.39	3 01

THE DISEASE

SYMPTOMS

To determine the character of the disease and its symptoms several hundred semiripe and ripe Smooth Cayenne fruits were cut into slices about 1 to 2 centimeters thick, and each slice was examined for internal discoloration. The infection is generally observed first in the trilobal fleshy placenta in the form of tiny spots or fine striations. The discoloration, which is best observed in cross sections, usually lies beneath the epidermis of the placenta and seldom penetrates it completely. It may extend to the other walls of the ovarial loculi, but as a rule it does not invade the interjacent tissues. It may involve, however, the entire fruitlet (Plate 2, fig. 2) and extend to the vascular bundles of the core when the infection is severe. A portion of the fruit, half of the fruit (Plate 1), or the whole fruit (Plate 2, fig. 1) may be infected, with the basal fruitlets more severely damaged than those on top.

In advanced cases of infection the color of the affected parts is dark brown to bone brown [3] or nearly black, and in cases of general infection affecting the entire fruit, or almost all the fruitlets, the fruit may remain firm and hard, the advanced

[3] The colors indicated here and elsewhere in this paper are those of Ridgway's Color Standards and Color Nomenclature. Washington (1912).

stage of ripeness as shown externally by the yellow rind notwithstanding. Crispness and hardness of the affected parts seem to be outstanding characteristics of early, more or less complete infection.

There are no clearly visible external signs of the disease. Close and careful examination of the very bad cases of rots will reveal, however, that a fruit which has been completely infected during its early development, as may be judged from the extent and intensity of the internal discolorations, generally manifests a dull uneven ripening color, and firm hard texture which may be felt if the fruit is cut or pressed hard in the hands.

Similarity to and difference from fruitlet brown-rot.—This disease has so many things in common with the fruitlet brown-rot(17) of pineapple that to a casual observer they appear as the same disease. These diseases appear more frequently together in the same fruit than separately. They attack the same parts of the fruit and invade the same tissues in a more or less identical manner, and both are caused by bacteria. There are a few dissimilarities, however, by which they may be distinguished from one another. As the names indicate the fruitlet black-rot has a decidedly darker internal discoloration than the fruitlet brown-rot and is, therefore, more objectionable from the canner's viewpoint. It is, besides, more serious and devastating than the brown-rot, thus entailing greater losses to the grower or canner. Furthermore, black-rot is caused by an entirely different bacterium, and has so far not been found to affect the native varieties. The only effective way of telling one from the other, however, is to isolate the causal organism.

THE CAUSAL ORGANISM

Isolations from semiripe and ripe fruits.—To determine the flora present in the affected tissues, isolations from fruits having infections of various stages of development and showing different degrees of discoloration, were made in tubes of glucose bouillion + 1. This was done in the following manner: The fruits that showed infection, while cutting in the field was being conducted for general observation, were washed in running tap water to free them from dirt, then set aside to dry. When they became dry, new slices were made from each with a butcher's stainless-steel knife previously sterilized by dip-

ping in alcohol and flaming over an alcohol lamp; all the necessary pathological notes were taken at the same time. The surface of the discolored tissues was later singed with a red-hot spatula. Under observance of all necessary precautions to avoid contamination, up to ten small blocks in duplicate were aseptically scooped out, one by one, with the help of a scalpel, which was sterilized by dipping in alcohol and flaming over a flame before and after each operation, and planted each in a tube of glucose bouillion + 1. From each tube that showed turbidity after 24 to 48 hours' incubation at room temperature (25° to 30° C.), dilution plates were prepared. Transfers of the various distinct colonies that developed thereon were then made on potato glucose agar + 1.

Isolations from green or maturing fruits.—It was noted in the preceding isolation experiment that semiripe fruits are nearly as badly infected as the fully ripe ones. This is in complete accord with general observation in the field and in the factory. Therefore, bacterial infection may take place at some time during the development of the fruit. To throw light on this phase of the problem it became necessary to cut green or maturing fruits of the Smooth Cayenne variety into slices 1 to 2 centimeters thick and to examine each carefully for internal discoloration. Notes were taken on the pathologic condition of each specimen studied, and isolations made according to the same procedure and technic used in the first experiment.

Results of isolations.—The results obtained from the preceding two series of experiments are shown in Tables 2 and 3; the first gives descriptions of the various types of discoloration found in 200 fruits out of 600 examined, and the second also gives descriptions of the various types of discoloration found in 83 fruits out of 300 examined.

As given in Table 2 two kinds of bacteria were isolated with great frequency—the white and the yellow. Some other microörganisms, such as *Penicillium*, yeasts, a pinkish bacterium, etc., were also isolated, but owing to their erratic occurrence were dropped from the list.

It is shown in Table 2 that 35.50 per cent of the 200 infected fruits that were examined yielded pure cultures of the white bacterium, 23.50 per cent yielded pure cultures of the yellow bacterium, and 41 per cent yielded both the white and the yellow bacteria, at times occurring in the same plantings and at other

TABLE 2.—*Showing bacterial flora most commonly found in 200 infected semiripe and ripe pineapple fruits.*

Fruits examined.	Description of discoloration.	Fruits affected by— White bacterium.	Fruits affected by— Yellow bacterium.	Fruits affected by— White and yellow bacteria.
20	Slight browning in scattered eyes with fine striations on placentæ.	6	6	8
20	Complete infection in the form of slight browning and hardening in the eyes with streaks on placentæ and brown bundles in the core.	7	5	8
20	Complete infection in the form of brown hard eyes with soft brown rot in placentæ.	7	5	8
20	Complete infection in the form of brown hard eyes with bone brown hard placentæ.	8	4	8
20	Complete infection in the form of bone brown hard eyes with bone brown hard, rather dry placentæ and brown bundles in the core.	8	4	8
20	Several brown hard eyes with few dark brown hard placentæ.	7	5	8
20	Brown hard eyes and dark brown placentæ on ½ to ⅔ of top of fruits.	8	6	6
20	Brown hard eyes and dark brown placentæ on ⅓ to ½ basal part of fruit.	8	4	8
20	Brown hard eyes and dark brown placentæ on one side of fruit, top to bottom.	8	4	8
20	Brown hard eyes at base with light brown streaks in fruitlets on top.	4	4	12
	Fruits infected.................. per cent..	35.50	23.50	41.00

times in different plantings, from the same fruit. It is further shown that on the average there are more cases of fruitlet rot associated with the white bacterium than with the yellow bacterium, and that the white bacterium is more or less constantly associated with bad, severe cases of infection. Finally, although, in general, the white bacterium is capable of causing more severe rots with darker shades of discoloration, as a whole, it produces a set of symptoms very similar to that caused by the yellow organism. This fact shows the impossibility of telling with certainty what particular kind of infection is found in a fruit by mere ocular examination.

Results of the isolations from green or maturing fruits as given in Table 3 are, in a general way, a confirmation of the first isolation tests—that the white and the yellow bacteria are the two organisms found mostly associated wtih the fruitlet rots.

TABLE 3.—*Showing bacterial flora most commonly found in 88 infected green or maturing pineapple fruits.*

Fruits examined.	Description of discoloration.	Fruits affected by—		
		White bacterium.	Yellow bacterium.	White and yellow bacteria.
47	Slight browning in scattered eyes with fine striations on placentæ.	20	12	13
11	Complete infection in the form of slight browning and hardening of the eyes with streak on placentæ and brown bundles in the core.	4	3	4
0	Complete infection in the form of brown hard eyes with soft brown rot in placentæ.	0	0	0
0	Complete infection in the form of brown hard eyes with bone brown hard placentæ.	0	0	0
0	Complete infection in the form of bone brown hard eyes with bone brown hard, rather dry placentæ and brown bundles in the core.	0	0	0
3	Several brown hard eyes with few dark brown hard placentæ.	1	1	1
0	Brown hard eyes and dark brown placentæ on $\frac{1}{3}$ to $\frac{3}{4}$ of top of fruit.	0	0	0
9	Brown hard eyes and dark brown placentæ on $\frac{1}{3}$ to $\frac{2}{3}$ basal part of fruit.	3	3	3
3	Brown hard eyes and dark brown placentæ on one side of fruit, top to bottom.	1	1	1
10	Brown hard eyes at base with light brown streaks in fruitlets on top.	3	4	3
	Fruits infected..........per cent..	40.97	28.91	30.12

Other microörganisms are as rare as they are erratic in occurrence and as such were discarded. These results differ from those of the first, however, in some particulars; namely, the infection in the latter is less severe with less-pronounced discoloration; and there are more cases of fruitlet rot associated with the white organism alone than there are of either the yellow alone or the white and the yellow in association.

In addition the following generalities may be deduced from the two series, first, that the infecting organisms apparently gain entrance into the placental cavity of the fruitlet some time during the development of the fruit or, to be more specific, during and after anthesis, either through the decaying flower parts or through fissures running from the eye cavity downward into the placental lobes and, in some instances, through the mechanical cracks generally present at the base of the eye

cavity, particularly among large fruits; second, that the white organism is more active and virulent than the yellow even during the early stage of infection; and, third, that drying, hardening, and crispness of the affected tissues seem to be typical characteristics of the disease resulting from early severe infection.

PATHOGENICITY

Inoculation experiments.—With a view to determining the organism responsible for the occurrence of the fruitlet black-rot disease of pineapple five series of inoculations were conducted in Santa Fe, Bukidnon Province, beginning June, 1929. Only the white bacterium was used in these experiments inasmuch as the rest of the microörganisms had been tested and found to be associated with a more or less different type of spots, as reported by Serrano(17) in a paper on the bacterial fruitlet brown-rot of pineapple in the Philippines.

Series 1.—In this series 20 Smooth Cayenne fruits adjudged to ripen in about 40 days were used. The inoculation was done in the following manner: Twenty uniform upright fruits in a double row running from east to west were selected and tagged consecutively from 1 to 20. The first fruit was then disinfected all over with a 1:1000 solution of mercuric chloride in 70 per cent alcohol. As soon as they were dry a check puncture on each of the five eyes lined in a vertical order from top to base and facing the east (1–A) was aseptically made with a sterile steel needle. The needle was sterilized before and after the punctures were made by dipping in alcohol and flaming over an alcohol lamp. In every case proper care was observed to make the puncture at a definite angle so as to avoid the flower bowl where various microörganisms, particularly *Penicillium* and other molds, generally härbor. Then, in exactly the same manner five other punctures were made on the five opposite eyes facing west (1–B), but introducing with each puncture a mass of 48-hour-old culture of the white bacterium. Upon completion all punctures on both sides of the fruit were sealed with paraffin wax to prevent the entrance of extraneous microörganisms. The next nine fruits were treated in exactly the same way, using one separate culture of the white bacterium for each. Thus the first part (1–A to 1–B) of the first series was completed.

The second part (1–C to 1–D) is essentially the same as the first part, except that the inoculation was made by means of a 10-cc hypodermic syringe, injecting 1 cc of sterile distilled water as check into each of the five ovarial cavities facing east (1–C) and 1 cc of sterile water suspension of each of the same batch of cultures of the white bacterium used in the first series as inoculum into each of the five opposite ovarial cavities facing west (1–D).

Series 2.—This is a duplicate of the first series except that the fruits used were adjudged to ripen about 30 days from date of inoculation. The same ten batches of cultures of the white bacterium were employed, being inoculated by needle punctures in the first part and by hypodermic syringe in the second part.

Series 3.—The same procedure as in the first two series was followed with fruits adjudged to ripen about 20 days from date of inoculation.

Series 4.—Fruits adjudged to ripen about 10 days from date of inoculation were subjected to the same procedure as in the first three series.

Results of inoculations.—The fruits were picked and brought to the laboratory as soon as signs of ripening were noticed. Following the line of inoculation punctures every one of them was cut into longitudinal halves for observation (Plate 3). In this way the effect of the supposedly sterile puncture as well as the puncture with the inoculum on the ovarial lobes could be plainly observed. Every puncture was traced and meticulously examined for any discoloration in the cavity as well as on the placental lobes, and for any change that might have taken place in the texture of such tissues. The results of these examinations are given in Table 4.

The results obtained from the first series of inoculation experiments as given in Table 4 (1–A to 1–D) furnish a strong indication that the white bacterium is responsible for the occurrence of the fruitlet black-rot disease. All of the inoculated punctures in each of the 20 fruits turned out 100 per cent very strongly positive, while the check punctures on the same fruits remained negative (Plate 3). Most of the characteristic features of the disease in nature have been more or less completely reproduced. Brown lesions with dark brown irregular margins are in abundance in the inoculated fruitlets. Gumming, brown spotting on the placental lobes with fine reddish-brown striations, and browning of the vascular bundles are found in the

infected tissues. Crispness and hardening of the infected parts as found in nature are the only features of the disease not fully reproduced by artificial infection. Relatively few spots have shown a decided tendency to become really crisp and hard. This notwithstanding, the white bacterium was always regained by reisolation from infected tissues exhibiting such varied types of discolorations, while the check punctures remained sterile.

TABLE 4.—*Showing results of inoculation experiments on 80 fruits adjudged to ripen in 40, 30, 20, and 10 days from date of artificial infection.*

[+, Positive; ++, strongly positive, +++, very strongly positive, —, negative; x, natural infection.]

Series.	Number of fruits.	Method of inoculation.	Inoculum.	Observations.
1-A	1-10	50 punctures	None	50—
1-B	1-10	do	White bacterium	50+++
1-C	11-20	50 injections	None	50—
1-D	11-20	do	White bacterium	50+++
2-A	1-10	50 punctures	None	40—, 10x
2-B	1-10	do	White bacterium	40++, 10+++
2-C	11-20	50 injections	None	50
2-D	11-20	do	White bacterium	43++, 7+++
3-A	1-10	50 punctures	None	50—
3-B	1-10	do	White bacterium	50++
3-C	11-20	50 injections	None	40 , 10x
3-D	11-20	do	White bacterium	50++
4-A	1-10	50 punctures	None	45—, 5x
4-B	1-10	do	White bacterium	45+, 5++
4-C	11-20	50 injections	None	50
4-D	11-20	do	White bacterium	40+, 10++

Spreading of the discoloration is rather limited, the browning being found only in the tissues immediately surrounding the punctures. There are instances, however, where spreading occurred in the form of brown bundles in the core and tiny dots and fine reddish brown striations in the placental lobes.

The section of the fruit, or the stage or ripeness of the fruit seems to have some influence on the development and spread of the discolorations. Inoculation punctures made towards the top of the fruit usually produce cavities with leathery reddish brown to dark brown walls. These cavities are generally found with gum, whereas the inoculation punctures made on the midsection of the fruit down the base produced the types of discoloration approaching most the appearance of naturally infected fruits. The brown vascular bundles are present in

abundance in some positive cases but confined to the inoculated side of the fruit. Such browning of the bundles is invariably found originating from the inoculated eyes (Plate 3).

The inoculation made with the hypodermic syringe (1–C, Table 4) produces discoloration which decisively confirmed the results from the needle-puncture inoculations. With few exceptions the set of symptoms produced is the same as, and no more nor less characteristic than, in the puncture inoculations. Cavities with brown corky walls develop in the inoculated eyes above the midsection of the fruit, while lower down the tissues are rather soft spots with varied types of discoloration. Brown lesions with dark brown irregular margins and water-soaked gray centers are in abundance. A profusion of brown bundles and grayish brown wet placentæ are also often found.

As in the needle-puncture series the white bacterium is recovered in every instance by reisolations from these different types of discoloration, while the sterile-water infections as check remain sterile. The ten different isolations of this white bacterium vary somewhat among themselves, as will be discussed later, but when inoculated into the fruits their pathogenicity could not be clearly differentiated.

The results of Series 2 (Table 4, 2–A to 2–D) are essentially the same as those of Series 1. The same thing may be said of the results obtained from Series 3 and Series 4 (3–A to 3–D, and 4–A to 4–D, Table 4) where all of the inoculated fruits showed positive to very strongly positive infection. That the white bacterium is a very aggressive parasite of the pineapple fruit was clearly demonstrated by the positive results obtained from Series 4 when the fruits were picked and cut open for examination 10 days after the date of inoculation, while the checks remained sterile. The only characteristic features of the disease in nature not observed in this series are crispness and hardness of the affected placental lobes. These symptoms are probably reproducible only when the infection takes place while the fruits are still green, for at this stage of fruit development the upsetting of the metabolic processes in the plant system by the invading pathogen has a better chance of being decisive than at any other time.

As in the preceding series the white bacterium is recovered by reisolation from the various types of discolorations produced by artificial infection, with the checks remaining sterile, except a few, which evidently have been naturally infected at the be-

ginning. All of these facts taken together would seem to prove that the fruitlet black-rot disease of pineapple is caused by the white bacterium.

Series 5.—After a more or less definite determination of the cause of the malady, the fifth and last series of inoculations was carried out in a seminatural manner as a confirmatory test. A block of twenty-four double rows (standard spacing, 56 by 22 by 18 inches) of Smooth Cayenne at bloom was selected for this experiment, May, 1930, in Santa Fe, Bukidnon Province. By the use of a compressed-air sprayer, row 1 was sprayed with tap water as check, and skipping 2 and 3 as blanket rows, row 4 was sprayed with water suspension of a three-day-old culture of the white bacterium, thoroughly wetting the inflorescences in both cases. In exactly the same manner and order, the remaining rows were treated at different intervals as follows: Rows 7 and 10, when the fruits were about two months old; rows 13 and 16, when the fruits were about three months old; and rows 19 and 22, when the fruits were about four months old and already maturing. Under these conditions of the experiment, the results may answer the question as to what particular stage of the fruit is most susceptible to the disease.

Results.—Fruits of the sprayed rows were picked and brought to the laboratory for observation as soon as signs of ripening were shown. As they were sliced, one by one, notes on their pathologic conditions were taken as presented in Table 5.

TABLE 5.—*Showing results of inoculations by spraying fruits of different stages of development with the white bacterium.*

Row.	Treatment.	Age of fruits.	Fruits observed.	Pathologic observations.				Total infection.	Infection due to inoculation.
				Healthy.	Slightly infected.	Severely infected.	Total loss.		
		Months.						*Per cent.*	*Per cent.*
1	Check	1	385	267	79	31	8	30.6	------
4	Inoculated	1	390	86	195	78	31	77.9	47.3
7	Check	2	387	252	89	35	11	34.9	------
10	Inoculated	2	389	97	195	74	23	75.0	40.1
13	Check	3	380	266	80	27	7	30.0	------
16	Inoculated	3	395	134	188	57	16	66.0	33.0
19	Check	4	391	262	86	33	10	32.9	------
22	Inoculated	4	386	178	157	41	10	53.9	21.0

Table 5 shows that the check rows gave a total infection of 30.6 per cent, 34.9 per cent, 30.0 per cent, and 32.9 per cent

with an average of 32.1 per cent; while the inoculated rows gave 77.9 per cent, 75.0 per cent, 66.0 per cent, and 53.9 per cent. The infections caused by the artificial inoculations are represented, therefore, by the difference between the two series, or 47.3 per cent, 40.1 per cent, 33.0 per cent, and 21.0 per cent. The positive results in the check were undoubtedly caused by natural infection.

It may be presumed that the pathogen gains access to the placental lobes of the fruit through decaying flower parts during the early stages of the development of the pineapple fruit, while during the later stages it may course its way in through other avenues besides, such as the mechanical cracks which are usually present at the bases of the three alternating stamens in large fruits, and the ruptures of the fissures running down from the eye cavity. In the light of these hypotheses it would seem reasonable to expect greater incidence of the disease from the fruits which have had the artificial inoculation during the latter part of their development than from those which had it earlier. The fact as presented in Table 5 is contrary to this expectation, however. There are evidently two possible explanations of this; to wit, (*a*) the flowers or the fruitlets composing the fruit have their bracts still open during the early stages and are thus easily vulnerable to the attack of any parasite like the white bacterium which may happen to alight on them, whereas during the latter stages such bracts are generally more or less tightly closed, thereby excluding a great number of the invading microörganisms; (*b*) the plants to which the inoculum was introduced earlier were given a longer exposure to infection than those sprayed later.

Recapitulating, these results conclusively confirm the results of previous inoculations, that the white bacterium is the primary and only cause of the pineapple fruitlet black-rot disease; that the disease could be reproduced by inoculating the fruit with the white bacterium even without the aid of artificial injury to the fruit; that infection can take place at almost any stage of the fruit, although its incidence decreases as the fruitlets become more mature and tighten their bracts closely together; and, that some plants are apparently very resistant if not immune to the infection as represented by those which, in spite of the profuseness of the artificial inoculation given them, remained healthy till maturity. Such individuals when proven true to type should verily constitute a more or less permanent solution of this problem.

MORPHOLOGIC CHARACTERS

This organism is a short white rod with rounded ends, occurring singly, but usually in pairs, and sometimes in short chains. The size varies with the age of the culture. Single rods from 24-hour-old cultures on beef extract dextrose agar plates measure 1.7 to 2.0 μ by 0.5 to 0.6 μ; those in pairs are 3.4 to 4.0 μ by 0.5 to 0.6 μ. Rods from 3-day-old and 6-day-old cultures were found to be smaller (Plate 5, figs. 3 and 4). The organism stains readily with most stains. It stains well with gentian-violet and with both concentrated and diluted (1:4) carbol-fuchsin. Carbol-fuchsin stains it in bands especially when the culture is rather old. It produces neither capsules nor spores. It is Gram-negative and not acid-fast. It is motile with 1 to 4 polar flagella, 3 to 4 times as long as the body, as shown by Plimmer's(14) method (Plate 5, fig. 2).

CULTURAL CHARACTERS

This study was carried out in the laboratories of the Bureau of Science with a view to identification of the causal organism. All cultures were kept at room temperature (25° to 30° C.) in the dark, under which conditions luxuriant growth of the organism, particularly on potato glucose agar + 1, was observed.

Beef-extract agar with 2 per cent dextrose.—This proved to be a favorable medium for the culture of this organism. Dilution plates produce colonies of about $\frac{3}{4}$ mm in diameter in 24 hours. In general the colonies are white with undulate to lobate edges, somewhat curled and finely granular; a few are not curled and have entire edges. The surface may be either smooth or rugose, radiately ridged, pulvinate to effuse. The submerged colonies are small and lenticular.

The growth is white at first but with the development of a pale greenish pigment it may assume an ivory-yellow color. The medium may be colored owing to the production of the pigment. The intensity of the pigment varies to some extent with the strain.

Colonies of certain strains are more or less constantly smooth, while others have a varying tendency towards rugosity. Rugosity seems to be accompanied by a dull appearance and smoothness by a glistening wet aspect. In the first few days the consistency is butyrous, becoming more or less viscid in 5 to 6 days, which is more marked in some of the strains than in others. There is, however, no apparent correlation between the varying characters of pigmentation, rugosity, or viscidity.

In slants moderate, filiform, flat, glistening growth develops generally with water of condensation after 24 hours. The streak spreads with a contoured, curly edge and wrinkling back of the margin. With age, say, 5 to 6 days later, the curly surface may turn smooth and glistening, and the mass of growth may ooze down to the base of the slant. The culture medium may be greened due to pigmentation.

Beef-extract agar.—This proved to be not as favorable a medium for the culture of this organism. The growth is scanty, and the colonies are considerably smaller. Except for feebleness the growth characters are as described in beef extract agar with 2 per cent dextrose.

Beef-extract broth.—The growth is feeble, producing slight cloudiness in 48 hours, and with flocculent surface growth and without sediment.

Beef-extract broth with 2 per cent dextrose.—Forty-eight hours after inoculation a thin wrinkled pellicle develops on the surface and the solution becomes slightly cloudy, but there is no precipitate. The pellicle is easily detached, dropping to the bottom and forming a flaky sediment. Dropping of the pellicle leaves a whitish ring attached to the glass at the surface. In 4 to 5 days the greenish pigment may be observed diffusing downwards from the pellicle.

Glycerine agar.—Forty-eight-hour colonies are pale ivory yellow, raised, smooth, with crenate edges; becoming barium yellow to straw yellow in 5 to 6 days; abundant, thick, rugose, and imparting strontian yellow to wax yellow pigment to the agar in two weeks, the color remaining apparently unchanged even after two months.

Potato-glucose agar + 1.—This proved to be a very favorable medium for the culture of the organism. Twenty-four hours after plating the colonies produced are about 1 mm in diameter, opalescent, convex, circular, entire, becoming pale greenish or ivory yellow, rugose, with crenate margin, and measuring 4 to 6 mm in diameter, after 2 days (Plate 4); abundant in 5 to 6 days, with colonies finally becoming smooth, round, creamy, wet, glistening, pulvinate to hemispherical, and imparting a pale greenish pigment to the medium (Plate 5, fig. 1). Rugosity and pigmentation vary somewhat with the strain. The medium may be greened due to the pigment.

In slants a filiform, flat, glistening growth with water of condensation develops in 24 hours. The streak spreads with a contoured, curly edge and wrinkling back of the margin. In

4 to 6 days the curly surface may turn smooth, glistening, starting from top to bottom, and the mass of bacteria may ooze down to the base of the slant (Plate 6). Simultaneously greening of the medium due to pigment production may be seen taking the same course.

Nutrient-gelatin stab.—Rapid stratified liquefaction occurs 24 hours after inoculation, accompanied by a slight production of greenish pigment and a heavy precipitate at the bottom. Liquefaction is complete after 2 days.

Milk.—A rennet curd with a layer of colorless whey on top is formed in 48 hours. Peptonization becomes apparent and is complete in 14 days.

Litmus milk.—A rennet curd is formed accompanied by peptonization. No trace of acid is observed even after 4 days, but litmus is slowly reduced and a yellowish whey is formed after a week.

Loeffler's blood serum.—Slight yellowish green growth develops on the surface of the slants 24 hours after inoculation. Liquefaction starts after 40 hours and is complete in 15 days.

Starch broth.—No diastatic action according to Eckford's(6) method; that is, the starch is not hydrolized.

Potato cylinders.—Rapid growth develops in 24 hours; abundant, glistening, with ivory yellow to cartridge buff after 4 days.

Pineapple juice.—The growth of the organism is fairly good on juice expressed from ripe fruit but scanty on juice from a mature or ripening fruit, the latter containing too much acid perhaps to support good growth.

Pineapple cylinders.—Unlike in the juice the growth is better on cylinders from mature or ripening fruit than on those from ripe fruit.

Cohn's solution.—Fairly good growth is produced with pellicles and little precipitate. The solution becomes turbid.

Uschinsky's solution.—Growth is manifested by slight cloudiness and slight precipitate in the solution.

CHEMICAL PRODUCTS FORMED

Fermentation of sugars.—Two per cent solutions of glucose, lactose, saccharose, salicin, mannite, dulcite, maltose, and xylose were prepared in triplicates with Dunham's peptone water and Andrade's indicator(19) in Smith's fermentation tubes. With these preparations three strains of the white bacterium were tested.

Glucose.—Twenty-four hours after inoculation cloudiness and deep rose pink to deep rose coloration are produced in the bulbs of the fermentation tubes by all of the three strains, indicating good growth and the production of acid from glucose. In 48 hours a pellicle is formed. In 14 days the deep rose coloration fades out and the reaction becomes alkaline, owing to the production of alkali from peptone. There is no trace of growth whatsoever and no gas in the closed arms of the fermentation tubes.

Lactose.—Cloudiness is observed in the bulbs of the fermentation tubes in 48 hours but no pellicles develop. No acid is shown until after 70 days, and no signs of growth and no gas production in the closed arms of the bulbs are shown by any of the three strains used.

Saccharose.—There is a very slight cloudiness in the bulbs of the fermentation tubes in 48 hours but no pellicle, and no acid even after 70 days. There is no growth and no gas in the closed arms of the fermentation tubes. The final reaction of the solution is alkaline, showing that alkali is produced from peptone.

Salicin.—Cloudy after 4 days. No pellicle, no acid, and no growth in closed arms of fermentation tubes. No gas even after 70 days.

Mannite.—Turbid in 48 hours, and with little sediment after 4 days. No acid till after 5 weeks, no sign of growth, and no gas in the closed arms of fermentation tubes.

Dulcite.—Turbid after 48 hours, but no acid, no pellicle, no gas, and no sign of growth in the closed arms of fermentation tubes.

Maltose.—Good growth in 48 hours, with thin pellicle but no acid, no gas, and no sign of growth in closed arms of fermentation tubes.

Xylose.—Very good growth in 48 hours, with pellicle and little acid production after 3 days, the medium turning coral red; no gas, and no growth in closed arms of fermentation tubes.

Nitrate reduction.—Following the method recommended by the Committee on Bacteriological Technic of the Society of American Bacteriologists,(4) tests were made to determine the nitrate-reducing power of ten strains of the pathogen. Marked differences in the amount of nitrate produced were shown by each in 24 hours incubation; namely, three of the strains giving a decisively positive reaction and the rest slightly so or not at all. After 6 days of incubation six of the strains produced a dark blood red color with the reagents, which soon turned brown-

ish with a flaky precipitate, and the rest a rose color without precipitate.

When grown in peptone broth (A), nitrate peptone broth (B), synthetic nitrate medium (C), and peptone broth with the addition of 2 parts per million of potassium nitrate (D), for 6 days, and tested for ammonia by the Hansen(9) method, it gives negative results in (A) and positive results in (B). These phenomena simply indicate nitrate reduction by all the strains. Positive tests for nitrate in (C) with negative results for same in (D) conclusively confirm this fact.

These results would seem to show that the pathogen is a nitrate-reducing organism and that its power as such varies with the different strains.

Hydrogen sulphide production.—None of the strains tested produce hydrogen sulphide as shown by Feller's(7) method.

Indol production.—Following the Gnezda(8) test, no indol production is shown by any of the strains.

PHYSIOLOGICAL REACTIONS

Relation to oxygen.—The agar shake culture method(4) has shown that the white bacterium is a strict aërobe.

Relation to temperature.—Very scanty growth at 7° to 10° C. Optimum temperature lies between 31° and 33° C. and the maximum between 43° and 45° C. Thermal death point lies between 51° and 53° C.

Relation to media.—Beef-extract broth with 2 per cent dextrose was prepared in 13 sets of 10 test tubes each, with the following reactions corresponding to each set in numerical order before and after sterilization: pH 3.0, 3.3, 3.6, 3.9, 4.2, 4.6, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0; and pH 3.14, 3.31, 3.63, 3.93, 4.36, 4.62, 4.98, 5.50, 5.93, 6.55, 7.06, 7.49, and 8.04. The pH values were determined by colorimetric method.(5)

Three strains of the white bacterium produced cloudiness after 24 hours incubation in culture having pH 4.36 to 7.49, and with distinct pellicles in cultures having pH 4.98 to 5.93, while others remained apparently negative. After 48 hours cultures having pH 3.93 and 8.04 showed slight turbidity, denoting scanty growth. Even after a week cultures having pH 3.14, 3.31, and 3.63 remained sterile, strongly indicating that the acid present is too much to allow any vegetative growth of the pathogen. From these results the optimum acidity for the pathogen would seem to lie between pH 5.0 and 6.0.

Relation to sugar.—Beef-extract broth was prepared in 7 sets of 10 test tubes each representing different amounts of sugars; namely, to the first set, no sugar added as check; to the second set, 3 per cent (2 sucrose and 1 dextrose); to the third set, 6 per cent (4 sucrose and 2 dextrose); to the fourth set, 9 per cent (6 sucrose and 3 dextrose); to the fifth set, 12 per cent (8 sucrose and 4 dextrose); to the sixth set, 15 per cent (10 sucrose and 5 dextrose), and to the seventh set, 18 per cent (12 sucrose and 6 dextrose). This proportion of sugars is similar to what was found in pineapple fruits (17) grown under natural conditions.

Three strains of white bacterium tested produced cloudiness after 24 hours incubation in all cultures except in the seventh which showed only very scanty flocculent growth on the surface of the liquid along the sides of the test tubes. The third exhibited by far the most luxuriant growth followed closely by the second and fourth, all with pellicles. The same order of development continued even after a week, which goes to show that about 6 per cent is the optimum sugar requirement for the best growth of the pathogen.

TECHNICAL DESCRIPTION

PHYTOMONAS ANANAS sp. nov.[4]

White rod-shaped type with rounded ends; occurring singly, but usually in pairs, and sometimes in short chains; size very variable depending on age—24-hour-old culture measuring on the average 1.8 μ by 0.6 μ—older cultures smaller; motile with 1 to 4 polar flagella, 3 to 4 times as long as the body; no capsules, no spores; Gram-negative and not acid-fast; readily stained with carbol-fuchsin and gentian-violet; a strict aërobe and capable of producing green pigment.

Agar colonies white, becoming ivory yellow, with undulate to lobate edges; somewhat curled and finely granular; a few are not curled and have entire edges; the surface may be either smooth or rugose, radiately ridged, pulvinate to effuse, becoming profuse, smooth, wet, glistening, pulvinate to hemispherical, and imparting a pale greenish pigment to the medium; consistency

[4] Following Bergey's Manual of Determinative Bacteriology (2) this pathogen is classed under *Phytomonas*, and the name *Phytomonas ananas* is proposed. None of the 77 species described by Burkholder (3) is identical with this. It should be *Pseudomonas ananas* under Migula's (11) classification, and *Bacterium ananas* if Smith's (18) classification is followed.

butyrous, becoming more or less viscid; submerged colonies small and lenticular; streak growth moderate, filiform, flat, spreading with a contoured, curly edge, wrinkling back of the margin and becoming smooth, glistening with age; profuse growth on potato-glucose agar + 1 and beef-extract agar with 2 per cent dextrose; in beef-extract broth with 2 per cent dextrose, wrinkled pellicles develop one after another, and soon settle to the bottom, forming a flaky sediment; the broth becomes cloudy but without precipitate; in 4 to 5 days a greenish pigment diffuses downward from the pellicle; liquefies nutrient gelatin and Loeffler's blood serum; forms soft curd in milk and litmus milk, accompanied by peptonization and formation of whey and alkali, litmus slowly reduced, but no acid formed; starch not digested or hydrolized; readily ferments glucose with production of acid but no gas; ferments also xylose, mannite, and lactose feebly, but not saccharose; reduces nitrate to nitrite; produces neither hydrogen sulphide nor indol; optimum temperature within 31° to 33° C.; thermal death point lies between 51° and 53° C.; grows best at about pH 5.5 with about 6 per cent sugar. The organism is pathogenic on pineapple, particularly the Smooth Cayenne variety.

Index number.—Following the chart recommended by the Society of American Bacteriologists (18) the index number is 5322–31124–2223.

PATHOLOGIC ANATOMY

As already mentioned in the discussion of the results of isolations, the internal discolorations resulting from this bacterial invasion have their origin traceable from the mechanical cracks generally present at the base of the eye cavity, particularly among large fruits, and the fissures running from the eye cavity downward into the placental lobes and ovarial loculi, thence into the tissues of the entire fruitlet and vascular bundles in the core, especially when infection is severe.

The pathogen may gain access to the placental cavity of the fruitlet sometime during the development of the fruit; namely, during and after anthesis either through the decaying flower parts, through ruptures of the fissures running from the eye cavity downward into the placental lobes, or through the mechanical cracks generally present at the bases of the three alternating stamens in large fruits. Such fruits are by nature possessed of large eyes with bracts seldom tightly closed even at maturity, thus remaining partly exposed to infection. That

greater pathologic infection is met with in this type of fruits than in others, seems to suggest that incomplete closing of the bracts of the eyes predisposes the pineapple fruits to all sorts of infections, most particularly to the fruitlet black-rot pathogen. Hence, fruits of this type are susceptible to the black-rot disease even after they have more or less fully developed.

Sections from the placentæ and core showing the typical symptoms of the disease in nature as well as those from positive inoculations were fixed in Carnoy's fluid, Flemming's solution, and formal-acetic-alcohol.

The method employed by Riker (16) in demonstrating the crowngall organism in its host tissue was used to advantage in staining the preparations. In addition many other stain combinations were tried, and carbol-fuchsin with a saturated solution of methyl-orange in clove oil as a counterstain was found to give the best results. With this combination the diseased cell walls and xylem elements stain red while the healthy cell walls stain yellow.

The intercellular spaces and the adjacent parenchymatous cells of the brown vascular bundles in the core are filled with granular refractive masses which readily stain with the nuclear stains. It is highly probable that these extraneous phenomena, not being found in healthy tissues, are masses of the pathogenic bacterium which has been invariably isolated from the duplicates of such materials.

SUMMARY

1. A bacterial fruitlet black-rot disease of the pineapple in the Philippines is described. The disease is one of the two major maladies of the pineapple, causing an infection of about 37 per cent on the average. It is found in all districts of the Archipelago where the Smooth Cayenne variety is grown.

2. The disease is characterized by dark brown to bone brown or nearly black discolorations in the placental lobes and placental loculi of one or more or all of the fruitlets which may involve the entire fruitlet or fruitlets and the vascular bundles of the core. It generally does not manifest itself externally and, like the brown-rot, is very difficult to diagnose without cutting the fruit. Very severely infected fruits are, however, distinguishable from the rest by being extraordinarily hard and having an uneven ripening color, even in an advanced stage of ripeness. It is evidently identical with the fruitlet black-rot disease of the pineapple in Haiti as reported by Barker.

3. The causal organism of the disease is a white bacterium hitherto unknown and is named *Phytomonas ananas* sp. nov. Inoculations with this white bacterium, with or without artificial injury to the fruit, have invariably reproduced the typical symptoms of the disease, conclusively establishing its pathogenicity. A technical description of the pathogen is given.

4. Evidence gathered would seem to suggest that the pathogen gets into the fruitlets during fruit development through decaying flower parts, mechanical cracks which are generally present in large fruits, and ruptured fissures running from the eye cavity downward into the placental lobes.

5. Some individual plants are apparently very resistant, if not immune, to the infection as represented by those which, in spite of the profuseness of the bacterial infusion sprayed on them, remained healthy till maturity.

LITERATURE CITED

1. BAKER, H. D. Fruitlet black-rot disease of pineapple. Phytopath. 16 (1926) 359–363.
2. BERGEY, D. H., et al. Bergey's Manual of Determinative Bacteriology. Ed. 3 (1930).
3. BURKHOLDER, W. H. The genus Phytomonas. Phytopath. 20 1 (1930) 1–23.
4. Committee on Bacteriological Technic of the Society of American Bacteriologists. Pure culture study of bacteria. Ed. 5 1 3 (1932).
5. Committee on Bacteriological Technic of the Society of American Bacteriologists. Pure culture study of bacteria, ed 5 1 3 (1933).
6. ECKFORD, MARTHA O. Thermophilic bacteria in milk. Am. Journ. Hyg. 7 (1927) 201–222.
7. FELLERS, C. R., O. E. SHOSTROM, and E. D. CLARK. Hydrogen sulphide determinations in bacterial cultures and in certain canned foods Journ. Bact. 9 (1924) 235–249.
8. GNEZDA, J. Sur les reactions nouvelles des bases indoliques et des corps albuminoides. Com. Rend. Acad. Sci. 128 (1899) 1584.
9. HANSEN, P. ARNE. The detection of ammonia production by bacteria in agar slants. Journ. Bact. 19 (1930) 223–229.
10. LUCAS, G. L. Pineapple growing in the West Indies. West Indian Bull. 8 (1907) 151–157.
11. MIGULA, W. System der Bakterien 1 (1897).
12. NOWEL, W. Diseases of Crop-plants in the Lesser Antiles. West Indies Committee, 14 Trinidad Sq., London (1923) 349–350.
13. PARK, H. P., and A. W. WILLIAMS. Pathogenic Microorganisms. Ed. 8 (1924).
14. PLIMMER, H. G., and S. G. PAINE. A new method for staining bacterial flagella. Journ. Path. and Bact. Edinb. 4 (1921) 286–288.
15. POLE-EVANS, I. B. Brown spot in pineapples. Report No. VI. Botany and Plant Path. Journ. Dept. Agr. Union So. Africa 9 (1924) 544.

16. RIKER, A. J. Some relations of the crowngall organism to its host tissue. Journ. Agr. Res. 25 (1923) 119–132.
17. SERRANO, F. B. Bacterial fruitlet brown-rot of pineapple in the Philippines. Philip. Journ. Sci. 36 (1928) 271–305.
18. SMITH, E. F. Bacteria in relation to plant diseases 1 (1905).
19. STITT, E. R. Practical Bacteriology, Blood Work, and Animal Parasitology. Ed. 7 (1923).
20. TRYON, H. Fruitlet core rot of pineapple. Queensl. Agr. Journ. 3 (1898) 458–467.

ILLUSTRATIONS

PLATE 1

Ripe Smooth Cayenne fruit cut longitudinally into halves, showing severe fruitlet black-rot infection on one side with brown bundles running from infected eyes; about × 1/3. (Photograph by C. S. Angbengco.)

PLATE 2

FIG. 1. Cross section of ripe Smooth Cayenne fruit showing severe fruitlet black-rot infection throughout; about × 1/2. (Photograph by C. S. Angbengco.)

2. Fruitlets of ripe Smooth Cayenne fruit showing different stages of fruitlet black-rot infection; about × 1. (Photograph by C. S. Angbengco.)

PLATE 3

Ripe Smooth Cayenne fruit cut longitudinally into halves following the line of needle puncture inoculations, showing characteristic browning of the infected fruitlets on one side in contrast with the unchanged natural cream color of the check fruitlets on the opposite side. Note also the brown bundles running from the infected fruitlets of the inoculated side; about × 1/3. (Photograph by C. S. Angbengco.)

PLATE 4

FIG. 1. Two-day-old plate culture of *Phytomonas ananas* sp. nov. on potato glucose agar + 1.

2. Same as fig. 1, enlarged; showing colonies with undulate to lobate, more or less curled edges, with either smooth or curled, radiately ridged, pulvinate to effuse, surface; about × 3. (Photograph by C. S. Angbengco.)

PLATE 5

FIG. 1. Six-day-old plate culture of *Phytomonas ananas* sp. nov. on potato-glucose agar + 1. Note that, in contrast with Plate 4, the colonies have become smooth, round, creamy, wet, glistening, pulvinate to hemispherical, with edges more or less entire, and forming somewhat transparent concentric rings; about × 3. (Photograph by C. S. Angbengco.)

2. *Phytomonas ananas* sp. nov.; smear preparation from 24-hour-old plate culture, stained by Plimmer's (14) method to show flagella. Note 1 to 4 polar flagella, 3 to 4 times as long as the body. Rods are mostly in pairs; about × 1,120. (Photomicrograph by F. B. Serrano.)

FIG. 3. *Phytomonas ananas* sp. nov.; smear preparation from 3-day-old plate culture, stained with dilute (1:4) carbol-fuchsin, showing decrease in size; about × 1,120. (Photomicrograph by F. B. Serrano.)

4. *Phytomonas ananas* sp. nov.; smear preparation from 6-day-old plate culture, stained with dilute (1:4) carbol-fuchsin, showing further decrease in size of individual rod, and its slow reaction to staining. Most of the bacteria especially those in chains stain in bands; about × 1,120. (Photomicrograph by C. S. Angbengco.)

PLATE 6

FIG. 1. *Phytomonas ananas* sp. nov., on potato-glucose agar + 1 slant culture; 2-day-old, showing condensation water.

2. Six-day-old slant culture, showing tear-drop ooze.

3. Sixteen-day-old slant culture, showing abundant viscid growth; the greened medium in *2* and *3* is due to pigmentation. (All photographs by C. S. Angbengco.)

PLATE 1.

PLATE 2.

PLATE 3.

PLATE 4.

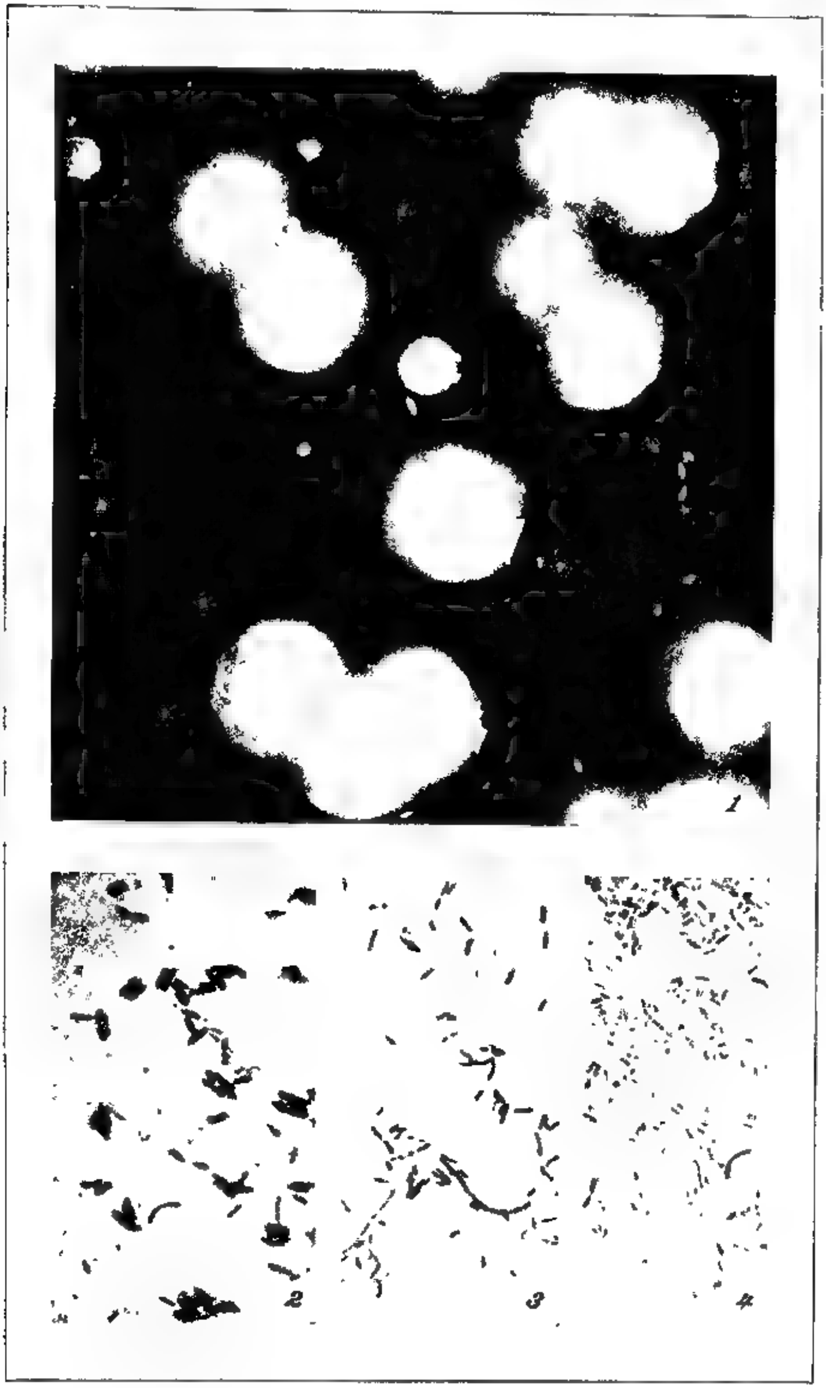

PLATE 5.

PLATE 6.

PINEAPPLE MEALY-BUG WILT IN THE PHILIPPINES[1]

By F. B. SERRANO[2]

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FIVE PLATES

INTRODUCTION

During the middle of 1927 the writer found a few Smooth Cayenne pineapple plants in a 1-year-old plantation at Calauan, Laguna Province, Luzon, suffering from "pineapple wilt." At the time the infestation was so mild that its possibility of becoming an important factor to reckon with in growing Smooth Cayenne pineapple seemed remote. About a year later, however, an entirely different aspect was presented by 2-year-old plantings belonging to the Philippine Packing Corporation in Makar, Cotabato Province, and in Santa Fe and Tangkulan, Bukidnon Province. Twenty to thirty per cent of both bearing and nonbearing plants were found collapsing from wilt. At about the middle of 1929, the disease broke out with alarming severity in several hectares of young plants grown from Hawaiian planting materials in Santa Fe, and caused almost a complete collapse of the entire fields soon after (Plate 1, fig. 4). Plants grown from Bukidnon seeds, on the other hand, were quite free from the infestation (Plate 1, figs. 1 to 3). This rather serious situation attracted the writer's attention and prompted this investigation.

The pest was perhaps introduced many years ago with the importation of Smooth Cayenne plants from Hawaii. But despite its already long standing in the Islands according to information obtained from old local pineapple planters, nothing has

[1] The second part of this paper dealing with the control measures will be published later in this journal.

[2] The writer wishes to express his thanks to the Philippine Packing Corporation for the splendid coöperation extended him in supplying practically all the materials and labor used in conducting the experiments. He is also grateful to Dr. E. Quisumbing, of the National Museum Division, for reading the paper and for encouragement in its preparation.

been done or written here on the subject. In Hawaii, where most of the canned pineapples sold in the world market are grown, the first reference to wilt was made by Larsen,(7) in 1910. In 1912 Higgins(5) expressed the opinion that the damage caused by wilt was limited to a few fields. A few years later it became so serious, however, that Illingworth(6) reported it as being responsible for the failure of the entire crop in 1920. Since then the wilt problem has been considered the most serious factor the pineapple grower has to contend with.

Sideris and Paxton(9) in summarizing the literature on root-rotting fungi with respect to plant failure expressed the belief that the root-rot caused by the phythiaceous organisms was probably one of the important factors responsible for the pineapple disease generally known as "wilt." Contrary to this, Illingworth(6) advanced the opinion that the pineapple mealy bug, *Pseudococcus brevipes* (Ckll.), was responsible for the transmission of the wilt disease by feeding on healthy plants after it has fed on diseased plants. He also stated that green spotting is a characteristic symptom of wilt which could be inherited from mother plants by vegetative propagation. Opinions vary, then, as to the true cause of pineapple wilt. For this reason and in view of the fact that the malady assumes alarming proportions and is of tremendous economic importance, this investigation seemed warranted. Carter,(1, 2) in 1933, published the results of his work on the same problem, which corroborate the present findings in practically all points.

DESCRIPTION OF THE MALADY [3]

"Wilt" is a general term which refers to that condition of a plant signifying flaccidity or lack of rigidity and freshness, with or without color changes in the affected tissues. In this particular case it is invariably associated with color changes, the extent of which depends somewhat on whether the wilt is of the "quick" or "slow" type. This "wilt" may be called "mealy-bug wilt" to differentiate it from "wilts" caused by other agents, like the phythiaceous fungi,(9) which are characterized by general wilting and yellowing of the leaves, accompanied by decay of the root system.

[3] This description is based on the results of artificial infestation experiments in order to tie up the types with their rather peculiar characteristics, which is not possible under field conditions.

QUICK WILT

This is the type of mealy-bug wilt resulting from a short period of feeding by a large number of mealy bugs. It usually occurs most prominently in young vigorously growing plants (Plate 2, figs. 1 and 2). In plants up to six or seven months old the tender leaves become pale, varying from very light dull green to pinkish or pale yellow. More characteristic than the color changes is the general loss of rigidity in the entire foliage, with the tender leaves becoming flaccid and bending outward. The tips of these leaves turn pale yellow, then become brown upon drying (Plate 2, fig. 2). The leaves of the inner whorls usually show green spots and may or may not have scattered small chlorotic areas with irregular margins (Plate 2, fig. 1). These two types of spotting (green and chlorotic) are usually seen on the same plant under field conditions.

With plants from nine to ten months old, there is a conspicuous reddening of the tender and medium-aged leaves. The outer leaves show either yellowish, pinkish, or yellowish brown color. Under field conditions, changes in color from pale green to pinkish or reddish shades are generally shown by plants widely spaced in the rows, while densely spaced plants show changes from pale green to yellowish or brownish shades, suggesting that light has some influence in the transformation. When quick wilt occurs at the flowering or fruiting stage, it results in a general yellowing or browning and drooping of the leaves, with the tips eventually drying up and showing welts with browned centers of secondary necrosis (Plate 3, figs. 1 and 2). As a result of this type of infection the undeveloped fruit may become "crooked-neck" and assume a more or less pendant position (Plate 3, fig. 1).

SLOW WILT

Differing somewhat from the preceding is the slow wilt occurring after a small colony of mealy bugs has ultimately developed into a large one. This type of mealy-bug wilt is generally found among not-too-vigorously growing plants. The color changes in this type are comparatively few. Its most outstanding symptom is perhaps the large number of mealy-bug feeding points generally covering the entire surface and interfering with the proper functioning of the leaves. The spotting is usually of the chlorotic type, although some green spotting may also occur. The tips of the leaves turn brown and dry, those of the older leaves bending outward and drooping, but none of the yellowish

or pinkish coloration characteristic of the quick wilt is in evidence. The younger leaves become flaccid although remaining upright, of a pale green color, the edges reflexing inward (Plate 1, fig. 4, X).

In both types of the mealy-bug wilt the roots are more or less collapsed, dried up, and generally invaded by saprophytic organisms. They are otherwise normal.

THE PINEAPPLE MEALY BUG AND THE WILT

The pineapple mealy bug, *Pseudococcus brevipes* (Ckll.), belongs to the order Homoptera and the family Coccidæ. It is parthenogenetic and viviparous. It develops rather slowly, two months at least being required by the youngest larva to develop into the gravid female. It is covered by a whitish waxy substance, which serves as a protection. It secretes honeydew, and has therefore almost invariably the attendance of two species of ants,[4] *Solenopsis geminata* Fabr. var. *rufa* Jerdon and *Pheidole megacephala* (Fabr.)

Fullaway(4) considered the insect to be partial to bromeliaceous and allied plants, and indigenous in Central and South America. In 1920 Morrison(8) reported it on pineapples and bananas in the Philippines.

On close examination hundreds of wilting specimens of 4- to 6-month-old Smooth Cayenne plants grown from Hawaiian planting material (mostly tops) during the early part of 1929 in Santa Fe, Bukidnon Province, were found infested more or less heavily by this insect, while apparently healthy-looking plants were practically free. Under field conditions the mealy bugs are mostly located on the tender parts of the leaves, generally in the crevices of the whorl. During sunny days they may be found all over the surfaces of the leaves, while during rainy days they usually congregate on the lower surface; in advanced cases of infestation they may penetrate as far down as the rootstock. These observations enhanced the suspicion that their presence has something to do with the incidence of pineapple wilt, hence the following experiments.

EXPERIMENT 1: WHAT CAUSES PINEAPPLE WILT?

Materials and methods.—Thirty-five empty gasoline cans were opened on the top and on one of the sides. The opening was made by cutting close to the seam all around the top and leav-

[4] Identified by Mr. Fidel del Rosario, assistant systematic entomologist of the Bureau of Science.

ing an inch-edge all around the cut side. Against the open side was placed a 1/8-inch-thick glass plate cut to fit the inside dimension of the can. The cans were filled with steam-sterilized plantation soil and placed on a bamboo flat form with posts covered with Tanglefoot to prevent ants and other insect from getting into the cans. Each of the cans was then planted with a healthy Bukidnon Smooth Cayenne top previously cured for three weeks in the sun and treated for mealy bugs by soaking in 1.0 per cent hot (45° C.) soapsuds for 30 minutes the day previous to planting; later the cans were divided into seven uniform groups.

February 1, 1929, or three months after planting, the plants of the first three groups were infested with the pineapple mealy bug (*Pseudococcus brevipes*), by placing one gravid female on each of the first group (1 to 5), ten gravid females on each of the second group (6 to 10), and twenty gravid females on each of the third group (11 to 15), while the fourth group (16–20) was not infested at all, as check. August 1, 1929, the remaining three groups of plants of the same age were infested with new batches of mealy bugs, also from wilted pineapple plants, in exactly the same way as in the first three groups.

Results.—Bimonthly observations were taken on the condition of each plant in the different groups until maturity as presented in Table 1.

Discussion of results.—Table 1 shows that all of the plants individually infested with twenty gravid females of *Pseudococcus brevipes* three months after planting showed typical symptoms of wilt two and one-half to six and one-half months after infestation (Plate 4, fig. 1); that of those infested individually with ten gravid mealy bugs, seven succumbed in six to twelve months, while of those infested individually with one gravid mealy bug only two became wilted in fourteen months. The greater the number of insects that feed on the plant at a time, the quicker is the wilt produced, and the more devastating becomes its effect. This may explain why in a field both quick wilt and slow wilt may be observed. On the other hand, all of those (16–20) that were not infested at all, as check, remained normal till maturity. Apparently, then, *Pseudococcus brevipes* is primarily and really the cause of this type of pineapple wilt.

The plants that had more insects at the beginning showed very many more a few months later; but when the plants became badly wilted only a few were left. Those that had but

one to start with were found with hundreds of them, literally covering the whole plants at the close of the experiment. This may perhaps be accounted for by the fact that the mealy bugs migrate to neighboring fresh material as soon as the host plants start wilting, and some of them may go down to the rootstock, especially during rainy days. Thus it is quite common to find under field conditions few or no mealy bugs on top of wilted plants while masses of them may inhabit the rootstocks.

TABLE 1.—*Showing Pseudococcus brevipes as the cause of pineapple wilt.*

[N, Normal; GS, green-spotted; W, wilting, typical; R, recovering; S, spindly; WF, wilted at fruiting; SF, spindly at fruiting; PRF, partially recovered at fruiting; NF, normal at fruiting.]

Group.	Plants.	Mealy bugs in each plant.	Condition of plants observed— Apr. 2, 1929.	June 2, 1929.	Aug. 1, 1929.	Oct. 2, 1929.	Dec. 1, 1929.
1	[a]1–5	1	4N, 1GS	4N, 1GS	4N, 1GS	4N, 1GS	4N, 1GS
2	[a]6–10	10	3N, 2GS	3N, 2GS	3N, 2GS	3N, 2W	2N, 3W
3	[a]11–15	20	2N, 3GS	2N, 3W	1N, 4W	5W	1R, 4W
4	16–20	([c])	5N	5N	5N	5N	5N
5	[b]21–25	1	----	----	----	3N, 2GS	3N, 2GS
6	[b]26–30	10	----	----	----	2N, 3GS	2N, 3GS
7	[b]31–35	20	----	----	----	2N, 3GS	2N, 3W

Group.	Plants.	Mealy bugs in each plant.	Condition of plants observed— Feb. 1, 1930.	Apr. 1, 1930.	Percentage of plants— Wilted.	Recovered.	Spindly.	Normal.
1	[a]1–5	1	4N, 1GS	4SF, 1WF	20	----	80	----
2	[a]6–10	10	1S, 4W	1SF, 4W	80	----	20	----
3	[a]11–15	20	1R, 4W	1PRF, 4W	100	(20)	----	----
4	16–20	([c])	5N	5NF	----	----	----	100
5	[b]21–25	1	3N, 2GS	2SF, 3NF	----	----	40	60
6	[b]26–30	10	2N, 3W	2SF, 3WF	60	----	40	----
7	[b]31–35	20	5W	1PRF, 4W	100	(20)	----	----

[a] Infested February 1, 1929. [b] Infested August 1, 1929. [c] Check.

Another feature noted aside from the chlorotic spots, as given in Table 1, is the production of green spotting on leaves of the majority of plants that wilted. The cause of this discrepancy among the wilted plants was not clearly understood. Hence, the mealy bugs present in all of the artificially infested plants were closely watched and examined as the experiment progressed in an attempt to correlate, if possible, their behavior and nature with the set of symptoms produced on their respective hosts. As a result, it was found that in plants where green spotting is present, the adult mealy bugs are of two colors, one

pinkish and the other grayish, whereas in plants where no green spotting occurs there is but one, the pinkish strain. The same thing was found to be true under field conditions. This fact would seem to be a clue to the solution of this phase of the problem. Following this line of evidence, another set of experiment was conducted.

EXPERIMENT 2: WHAT CAUSES GREEN SPOTTING?

Materials and methods.—In order to determine whether or not the gray strain of the pineapple mealy bug referred to in the preceding experiment has anything to do with green spotting, the following experiment was set April, 2, 1929. Fifteen potted plants were prepared in exactly the same manner as in Experiment 1, except that green spotted tops of Hawaiian Smooth Cayenne were substituted for the nonspotted tops of Bukidnon Smooth Cayenne. The plants were divided into three uniform groups. The first group (1 to 5) was infested individually with fifteen pink gravid mealy bugs, the second group (6 to 10) was left untouched, as check, while the third group (11 to 15) was infested individually with fifteen gray gravid mealy bugs.

TABLE 2.—*Showing that gray Pseudococcus brevipes causes green-spotting.*

[N, Normal; GS, green spotted; W, wilting, typical; R, recovering; WF, wilted at fruiting; PRF, partially recovered at fruiting; D, dead; NF, normal at fruiting.]

Plant group.	Mealy bugs in each plant.		Condition of plants observed—							Total percentage of plants—			
	Number.	Color.	June 2, 1929.	Aug. 2, 1929.	Oct. 1, 1929.	Dec. 1, 1929.	Feb. 2, 1930.	Apr. 2, 1930.	June 2, 1930.	Wilted.	Green-spotted.	Recovered.	Normal.
I	15	Pink	N	N	N	N	W	W	WF				
	15	do	N	N	W	W	W	W	D				
	15	do	N	W	W	W	R	R	PRF	100	0	20	0
	15	do	N	N	N	N	W	W	WF				
	15	do	N	N	N	W	W	W	D				
II	(a)		N	N	N	N	N	N	NF				
	(a)		N	N	N	N	N	N	NF				
	(a)		N	N	N	N	N	N	NF	0	0	0	100
	(a)		N	N	N	N	N	N	NF				
	(a)		N	N	N	N	N	N	NF				
III	15	Gray	GS	W	W	W	R	R	PRF				
	15	do	GS	W	W	W	W	W	D				
	15	do	GS	GS	GS	W	W	W	WF	100	100	20	0
	15	do	GS	GS	W	W	W	W	D				
	15	do	GS	W	W	W	W	W	D				

[a] Check.

Results.—Table 2 gives the results of bimonthly observations made for seven consecutive times on every plant of each group.

Discussion of results.—It is shown in Table 2 that in about six months all plants infested by the gray mealy bugs developed green-spotting and typical wilt symptoms, while those infested by the pink mealy bugs showed wilt symptoms alone two months later. On the other hand, the check plants developed neither typical wilt symptoms nor green-spotting. No green spots besides those that they originally had before planting were found at the close of the test. These results show that green-spotting is caused by the gray mealy bug, and that green-spotting does not constitute an important character of pineapple wilt, inasmuch as typical wilt could be reproduced with or without green-spotting. Its occurrence appears to be purely incidental and inherent in the presence of the gray strain of the pineapple mealy bug. It seems certain, however, that wherever green-spotting is found in abundance wilting is fast and decisive. Green-spotting would appear, therefore, to be more closely associated with quick wilt than with slow wilt.

In the pink mealy-bug series the main feature, aside from the general wilting of the plants, is the presence of chlorotic spots on the leaves, which are generally of very variable size and irregular margin but may be circular and minute at times. Where large colonies have developed the leaves are more or less covered with such rugose, somewhat translucent, chlorotic areas with brown necrotic centers. Stained sections through these areas show degenerated chloroplasts, the thickening of the cell walls, and the absence of starch. On the other hand, in the gray mealy-bug series the outstanding characteristic in addition to general wilting is the prevalence of green-spotting as previously stated. These green spots, which at first appear as faint yellowish green homogenous spots, arise from the feeding point of the insects near the junction of the green and the white tissue at the proximal end of the leaf. Some of the green spots have a concentric zone of lighter green around the darker center. These eventually become green welts, which are slightly raised in older tissue and particularly conspicuous in chlorotic leaves that have dried and shrivelled as commonly found obtaining under field conditions. Stained sections through these green spots show neither degeneration of the chloroplasts nor thickening of the cell walls, as typically seen in the chlorotic areas, but reveal instead an increase in the size and number of the chloroplasts.

That the check plants showed no sign of wilt or new green spots up to the end of the experiment, despite the fact that they had originally green spots in abundance, disproves Illingworth's (6) claim that green-spotting is a typical wilt symptom which could be inherited from mother plants by vegetative propagation. The writer believes that green-spotting is a localized disturbance brought about by the toxic effect of the secretion produced by the gray strain of the mealy bug, which is probably chemically distinct from that of the pink strain. Comparative microchemical study of the feeding areas of these two strains should prove elucidating. This belief seems to be further strengthened by the fact that repeated isolations made from these feeding points—namely, green spots and chlorotic areas—so far failed to show that there is any specific microörganism associated with their occurrence.

During the course of the experiment it was found that the green-spotting strain establishes larger colonies in a shorter lapse of time than the nongreen-spotting strain, and that the former changes the feeding points on the leaf oftener than the latter. This being the case, it is not surprising to see plants infested by this green-spotting strain wilting quicker than those infested by the other strain.

Another feature observed is the change in color taking place during the life of the mealy bug. This change seems to determine the mealy bug's individual capacity for toxicity as it in effect determines its power of producing green spots. It was noted that both the gray and the pink gravid mealy bugs produce young of exactly identical pinkish color, which makes it next to impossible to differentiate one from the other until they become almost mature, at which stage the green-spotting strain turns gray, while the nongreen-spotting strain remains pink throughout.

It was also noted that the development of mealy-bug colonies in all of the cultures under control is not as rapid and vigorous as under field conditions where there is free attendance of the two species of ants, *Solenopsis geminata* Fabr. var. *rufa* Jerdon, and *Pheidole megacephala* (Fabr.), which feed on the honeydew secreted by the mealy bugs. In view of the fact that the rate of wilting of the pineapple plant depends a great deal on the number of mealy bugs that simultaneously feed on it, it is understandable that wilting of plants under the controlled experiments proved to be not as sudden as in the field.

EXPERIMENT 3: RECOVERY FROM WILT

Materials and methods.—Pressed by the great need for planting material and the tremendous collapse due to wilt of several hectares of new plantings in Santa Fe, Bukidnon, it was decided to find out if wilting plants could be revived. August 1, 1929, thirty wilting plants showing uniformity in size, age, stage of wilting, and volume of mealy-bug infestation were collected and trimmed by pruning all top leaves as well as the butt and roots. They were then divided into two equal lots, the first lot treated with 1.0 per cent hot (45° C.) soap solution for mealy-bug control, and the second lot left untreated as check. Both were cured under the sun by piling them separately, butts up. After curing for five days five plants from each lot were planted singly as in the preceding experiments. In like manner the remaining twenty plants were planted singly in two batches of ten after curing for fifteen and thirty days, respectively.

Results.—Observations similar to those taken on the preceding two experiments were taken as shown in Table 3.

TABLE 3.—*Showing recovery of wilted plants.*

[GS, green-spotted; S, spindly; R, recovering; W, wilting, typical; SF, spindly at fruiting, RF, recovered fully at fruiting.]

Group.	Plants.	Treatment.	Length of curing.[a]	Condition of plants observed— Oct. 1, 1929.	Dec. 1, 1930.	Feb. 2, 1930.	Apr. 2, 1930.	June 2, 1930.
			Days.					
1	1–5	None	5	2GS, 3S	2GS, 3S	2GS, 3S	3W, 2S	4W, 1S
2	6–10	Soapsuds	5	5S	2R, 3S	5R	5R	5R
3	11–15	None	15	2GS, 3S	2GS, 3S	2GS, 3S	3W, 2S	4W, 1R
4	16–20	Soapsuds	15	5S	3R, 2S	5R	5R	5R
5	21–25	None	30	2GS, 3S	2GS, 3S	2GS, 3S	3W, 2R	3W, 2R
6	26–30	Soapsuds	30	5S	4R, 1S	5R	5R	5R

Group.	Plants.	Treatment.	Length of curing.[a]	Condition of plants observed— Aug 2, 1930	Oct. 2, 1930.	Percentage of plants— Wilted.	Recovered.	Spindly.
			Days.					
1	1–5	None	5	4W, 1S	4W, 1SF	80	----	20
2	6–10	Soapsuds	5	5R	5RF	----	100	----
3	11–15	None	15	4W, 1R	4W, 1RF	80	20	----
4	16–20	Soapsuds	15	5R	5RF	----	100	----
5	21–25	None	30	3W, 2R	3W, 2RF	60	40	----
6	26–30	Soapsuds	30	5R	5RF	----	100	----

[a] By curing is meant trimming and drying in the sun of planting materials before they are set in the soil to prevent spoilage.

Discussion of results.—Table 3 shows that all of the wilting plants when pulled up, trimmed and cured, and dipped in soap solution for mealy-bug control and then planted, could recover and produce healthy good-sized fruits; while those treated similarly but not dipped in soap solution continued to wilt, excepting one in the first group which became spindly, one in the third group, and two in the fifth group which fully recovered and bore healthy fair-sized fruits. These results seem to indicate that wilted plants could be revived by proper treatment; that freedom from the pineapple mealy bug is the key to a successful treatment, and that this could be attained fully by hot soapsuds treatment alone and partly by proper curing in the sun. The longer the material is cured the greater is its chance of recovery from wilt when planted. This has been demonstrated repeatedly by mass planting under field conditions (Plate 4, fig. 2). That this is so may be explained by the fact that it has been observed that under natural conditions the mealy bugs abandon wilting plants because of lack or insufficiency of suitable food; hence, the recovery of plants occasionally observed under field conditions. For the same reason, aside from want of shelter, they also abandon infested planting material that is undergoing the curing process.

All of the plants used originally had green spots in addition to typical wilt symptoms when planted; but during the course of their growth up to maturity, only four out of fifteen developed green-spotting—two in the first group, one in the third group, and one in the fifth group. Those that showed green-spotting when examined proved, however, to have gray and pink mealy-bug infestation, not having been treated with soapsuds at all, which simply indicates that the presence of the gray mealy bug is essential in the production of the green spots.

It may also be seen from the same table that instead of quick wilt as generally observed in previous experiments, slow wilt is the rule, appearing not earlier than eight months after planting and from which no recovery seems possible. This may be explained by the fact that the infestation started with comparatively small mealy-bug colonies on quite slow-growing plants. According to field observations and the results of Experiments 1 and 2, only simultaneous action of a large number of mealy-bug colonies on succulent, vigorously growing plants can produce quick wilt.

In confirmation of the results obtained from the two preceding experiments, it is definitely shown that *Pseudococcus bre-*

vipes is primarily and wholly the cause of pineapple mealy-bug wilt.

EFFECT ON FRUITS

At harvest time when infestation is heavy the pineapple mealy bugs are found in large numbers on fruits, stems, and sometimes on slips. The fruits become unclean-looking primarily because of the presence of honeydew secreted by the mealy bugs which becomes moldy (Plate 5). Carter and Ito (3) have the following to say in concluding their report on the matter: "The results clearly indicate that the presence of mealybugs in large populations at the base of fruits considerably reduces the quality of fruit by rendering the basal slices unmarketable as well as increasing the number of culls due to leaking and fermentation."

SUMMARY

1. A disease called "pineapple mealy-bug wilt" is described. It has been found in the Philippine Islands wherever the Smooth Cayenne variety is grown. It appears to be identical with the wilt reported from Haiti and Hawaii, whence it might have originated through the introduction here of planting material.

2. Pineapple mealy-bug wilt is characterized by a general wilting of the plant, with or without green-spotting on the leaves. It assumes a number of forms depending upon the age and succulence and vigor of the plant, as well as the size and time of the onset of the initial mealy-bug infestation. A large population at the onset of the initial mealy-bug infestation produces quick wilt, while a small number produces slow wilt. The younger and more succulent and vigorous the plant, the quicker it succumbs to quick wilt.

3. Infestation experiments have conclusively shown that pineapple mealy-bug wilt is primarily and truly caused by the pineapple mealy bug, *Pseudococcus brevipes* (Ckll.). The insect evidently secretes a nonliving toxic principle, which causes the wilting of the plant, producing typical wilt symptoms in about two months.

4. Plants affected by quick wilt may recover and produce small fruits, which are otherwise normal. Slow-wilt victims do not seem to be able to recover at all.

5. The abundance and general vigor of the mealy-bug colonies seem to be greatly favored by the attendance of two species of ants, *Pheidole megacephala* (Fabr.) and *Solenopsis geminata* Fabr. var. *rufa* Jerdon.

6. There are two strains of the pineapple mealy bug, the gray and the pink. The former produces green spotting which is not an important characteristic of wilt though very common among quick-wilt cases, while the latter produces chlorotic spots that are characteristic of the two types of wilt but more commonly met with in slow wilt. Both spots are the localized effect at the insect's feeding point.

7. The ability to produce green spots is inherited by the young from the parent gray mealy bug.

LITERATURE CITED

1. CARTER, W. The pineapple mealybug, Pseudococcus brevipes and wilt of pineapples. Phytopath. 23 3 (1933) 207–242.
2. CARTER, W. The spotting of pineapple leaves caused by Pseudococcus brevipes, the pineapple mealybug. Phytopath. 23 3 (1933) 243–259.
3. CARTER, W., and K. ITO. Some effects of Pseudococcus brevipes (Ckll.) on pineapple fruit. Proc. Hawaiian Ent. Soc. 8 (1932) 37–39.
4. FULLAWAY, D. T. Insects affecting pineapple production. Second annual short course in pineapple production. University of Hawaii (1923) 52–61.
5. HIGGINS, H. E. The pineapple in Hawaii. Hawaii Sta. Press Bull. 36 (1912).
6. ILLINGWORTH, J. F. Preliminary report on evidence that mealybugs are an important factor in pineapple wilt. Journ. Econ. Ent. 24 (1931) 877–889.
7. LARSEN, L. D. Diseases of the pineapple. Hawaiian Sugar Planter's Assoc. Path. Phys. Ser. Expt. Sta. Bull. 10 (1910).
8. MORRISON, H. The nondiaspine Coccidæ of the Philippine Islands, with descriptions of apparently new species. Philip. Journ. Sci. 17 (1920) 147 202.
9. SIDERIS, C. P., and G. E. PAXTON. Pathological, histological, and symptomatological studies on pineapple root rots. Am. Journ. Bot. 18 (1931) 465–498.

ILLUSTRATIONS

PLATE 1

FIG. 1. Three-month-old Smooth Cayenne pineapple plants free from wilt; about × 1/50.

2. Eight-month-old Smooth Cayenne plants free from wilt; about × 1/50.

3. Thirteen-month-old Smooth Cayenne plants free from wilt; about × 1/50.

4. Thirteen-month-old Smooth Cayenne plants on the verge of collapse due to wilt. Plants in front are victims of quick wilt, while those marked with x are suffering from slow wilt; about × 1/50. (All photographs by F. B. Serrano.)

PLATE 2

FIG. 1. Four-month-old Smooth Cayenne plant suffering from quick wilt, showing green-spotting and chlorotic areas on top leaves; about × 1/5.

2. Six-month-old Smooth Cayenne plant affected by quick wilt; about × 1/6. (Photographs by C. S. Angbengco.)

PLATE 3

FIG. 1. Fifteen-month-old Smooth Cayenne plant succumbing to quick wilt at fruiting, showing crooked-neck malformed fruit; about × 1/8.

2. Leaves from fig. 1, enlarged to show green-spotting and chlorotic areas caused by the pineapple mealy bugs' feeding; about × 1/2. (Photographs by C. S. Angbengco.)

PLATE 4

FIG. 1. Five-month-old Smooth Cayenne plants showing quick wilt in about two months as a result of artificial infestation with 20 gravid mealy bugs; about × 1/5.

2. Six-month-old Smooth Cayenne plants grown out of wilting stock by proper treatment; namely, curing and soapsuds dip; about × 1/20. (Photographs by F. B. Serrano.)

PLATE 5

Fifteen-month-old Smooth Cayenne plant with immature fruit showing many mealy bugs. The plant looks normal despite the prevalence of the mealy bugs. This is a case in which the initial infestation started with a small colony; about × 1/6. (Photographed by V. Ferrer.)

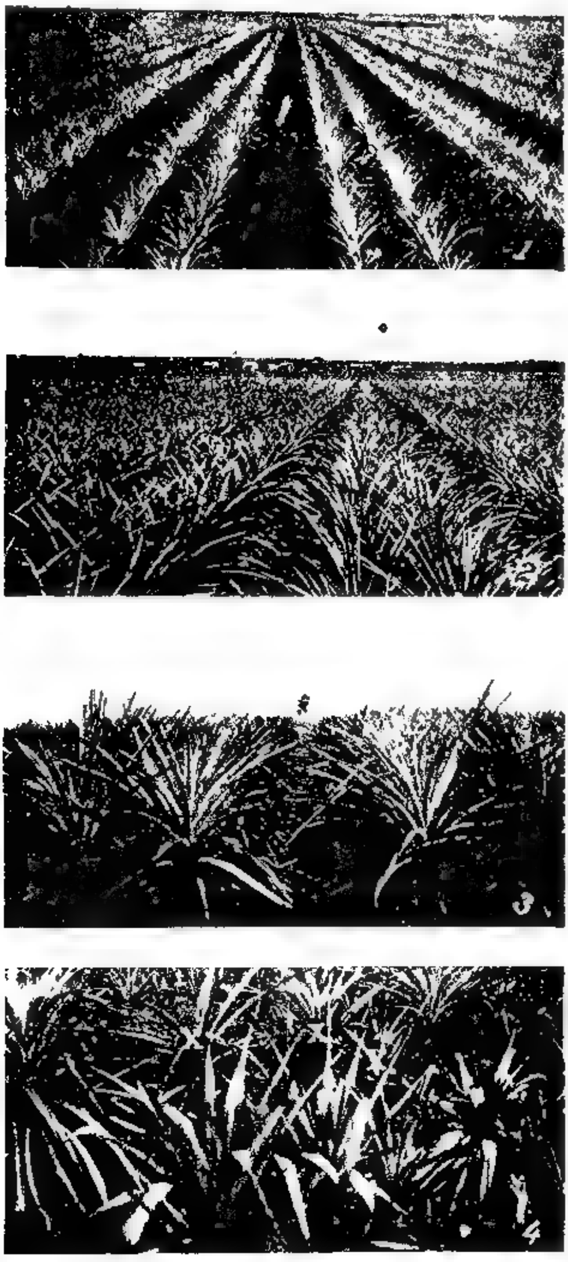

PLATE 1.

PLATE 2.

PLATE 3.

PLATE 4.

PLATE 5.

NEW LONGICORN BEETLES FROM THE JAPANESE EMPIRE, II (COLEOPTERA: CERAMBYCIDÆ)

By J. LINSLEY GRESSITT

Of Tokyo, Japan

The present paper is the second of a series, the first of which appeared in the Pan-Pacific Entomologist.[1] The six species here described are from Japan, the Loochoo Islands, and Formosa, the material having been collected by Mr. M. Kato, Mr. Y. Yano, and the author. I am indebted to Dr. E. C. Van Dyke, Mr. E. P. Van Duzee, and Mr. M. Kato, for their coöperation.

CERAMBYCINÆ

Genus CERAMBYX Linnæus

CERAMBYX MINUTUM Gressitt sp. nov.

Minute, narrow, subparallel. Black, clothed with sparse golden-gray pubescence, which is sparser on head, prothorax, and first three antennal segments; tarsi brown, densely clothed with golden-brown pubescence, paler above.

Head slightly shorter than prothorax, rugulose, sulcate between the eyes and antennal supports, the sulcature dividing on frons, forming a broad Y; clypeus and labrum short; palpi reddish brown; eyes prominent, rounded, grossly faceted. Antennæ silvery; scape rugulose, twice as long as broad; second segment minute; third segment nearly twice as long as scape; fourth segment slightly longer than scape; fifth segment equal in length to sixth, seventh, eleventh, and second and third together; eighth, ninth, and tenth segments subequal, successively shorter; eighth segment equal to third; third segment swollen at apex, fourth less so; last three segments flattened. Prothorax long, narrow, rather slightly swollen in middle, slightly narrower than elytra at base, rugulose dorsally, with an unbroken mid-longitudinal ruga. Scutellum small, rounded, clothed with golden pubescence. Elytra narrow, parallel-sided, rounded at apices; surface even, minutely granulose; scales greenish gold, giving to the black surface a mouse gray appearance. Ventral surface evenly

[1] Pan-Pac. Ent. 9 No. 4 (1933) 163–170.

clothed with scales below, with a silvery-gold tinge. Legs grayish black, the pubescence giving a silvery tinge; relatively long, the femora rather thin; tibiæ four-fifths as long as femora; tarsi four-fifths as long as tibiæ; first segment of fore tarsi barely longer than second or third segments, first segments of middle and hind tarsi as long as second and third tarsal segments together. Last segment of hind tarsi, including claw, nearly as long as first segment.

Length, 12 millimeters; breadth, 2.25.

Holotype, a unique specimen, probably a female, in the author's collection, taken at Kamikochi, Japan Alps, altitude 5,000 feet, on the main island of Japan, in August, 1932, by Y. Yano.

Genus EUSTRANGALIS Bates

EUSTRANGALIS VIRIDIPENNIS Gressitt sp. nov.

Body straw-colored; elytra totally green, shiny; middle and hind tibiæ and tarsi nearly black, fore tibiæ lighter; eyes and posterior portion of head black, except for a narrow oval spot of dark straw color on occiput; anterior and posterior lateral and ventral margins of prothorax black; pronotum and scutellum entirely of a shiny dark straw color; antennæ variable; scape dark straw-colored, succeeding segments gradually darker, distal segments nearly black; ventral surface of body entirely straw-colored.

Body naked, except for short, sparse, golden hairs on elytra. Head very finely punctate; prothorax impunctate, glossy; elytra finely and evenly punctate. Antennæ reaching to apical fifth of elytra; scape slightly curved and thickened towards apex, as long as third segment; third segment one and one-half times as long as fourth; third and fifth equal; sixth and succeeding segments slightly shorter, subequal; third to fifth segments fine, thickened at apices; sixth to eleventh thicker, subcylindrical; apical segment blunt. Prothorax rounded at sides in middle, constricted before and after middle. Elytra with humeral angles slightly rounded; external and sutural angles of truncature toothed, the tooth of the external angle much more prominent. Ventral surface of body naked, glossy; sterna finely punctate.

Length, 15 to 16 millimeters; breadth, 3.5 to 4.

Holotype, female, No. 50565, United States National Museum, taken near Taiheizan, Formosa, altitude 5,000 feet, May 9, 1932; allotype, male, in author's collection, taken near Arisan, Formosa, altitude 7,400 feet, June 5, 1932; one paratype taken with holotype at type locality, and three paratypes taken at Arisan,

June 4 and 5, 1932; all collected by the author. Paratypes in the collection of Mr. E. Gorton Linsley and that of the author. Most of the examples were found on cut surfaces of logs, probably of *Chamaecyparis formosensis*. The two specimens from Taiheizan have the elytra bluish green, instead of green, and the antennæ largely fulvous.

This species agrees very closely in structure with *Eustrangalis distenoides* Bates,[2] but is quite distinct. The latter has each elytron straw-colored and marked with a black longitudinal stripe from humerus to apex, instead of being green, and in having two black discal spots on the pronotum. *Eustrangalis distenoides* also differs in having the antennæ entirely black, the scape less curved and more angulate at apex, the tibiæ largely light-colored, and the last segment of the maxillary palpi dark. The elytra of *E. distenoides* are more attenuate, more sparsely clothed with hairs, and the humeral angles are more acute than in *E. viridipennis*. Furthermore, the last abdominal segment of the former is black.

Genus STRANGALIA Serville

Subgenus STRANGALINA Aurivillius

STRANGALINA GRACILIS Gressitt sp. nov.

Similar to *Strangalina attenuata* (Linn.). Narrow, elongate; antennæ fine, legs long; clothed with short, dense, golden pubescence, very thin on abdomen; very distinctly marked with three elytral bands and two, oblique, longitudinal lines on pronotum.

Coloration tawny testaceous brown, nearly golden on elytra, marked with black in the following manner: Apical segment of each antenna, maxillary palpi, genæ, and eyes black; a transverse black band between eyes, interrupted at midline of occiput; a lateral black stripe on each side of neck, and two finer ones on ventral side; prothorax very narrowly margined anteriorly and posteriorly and striped laterally with black, disk marked with two narrow oblique lines approximate posteriorly and diverging anteriorly; scutellum black; elytra marked with three transverse fasciæ dividing the elytra into four subequal parts, the last the longest; suture and extreme apical tips black; lateral surfaces of meso- and metathorax partially black, metacoxæ and posterior third of each abdominal sternum black; apices of mesotibiæ, mesotarsi, posterior halves of metafemora and metatibiæ, and metatarsi black.

[2] Linn. Journ. Zool. 18 (1884) 222, pl. 1, fig. 4.

Head long and narrow; surface very minutely punctate; frons subparallel-sided, flat; neck abruptly constricted directly behind eyes; eyes globular, prominent, antennæ fine, posterior segments very slightly thickened, reaching slightly beyond middle of elytra, scape three times as long as thick, shorter than third segment and subequal to fourth and fifth, second segment shorter than broad, sixth and seventh subequal in length, and eighth to eleventh subequal. Prothorax longer than broad, twice as broad at base as at apex; sides nearly straight, very slightly swollen; external basal angles acute; base four-fifths as broad as base of elytra; surface micropunctate. Scutellum small, triangular. Elytra long, narrow, slightly arched, straight-sided, clothed with very short golden and black bristles and very minutely punctate; apices narrow and subobliquely truncated. Ventral surface smooth, punctation nearly invisible; thorax pubescent; abdomen subglabrous, shiny, narrow. Legs long, hind femora reaching to elytral apices; tibiæ equal in length to femora; hind tarsi longer than femora or tibiæ; first segment of middle tarsi slightly shorter than remaining segments together; first segment of hind tarsi equal in length to remaining segments together; hind tarsi extremely narrow.

Length, 14 millimeters; breadth, 3.2.

Holotype, female, a unique specimen, in the author's collection, taken at Gusuku, Amami-Oshima Island, Loochoo Islands, July 10, 1932, by the author.

This interesting species is almost indistinguishable in markings and general appearance from the North American *Ophistomis luteicornis* (Fabr.), although it is more closely related in structure to the Palæarctic *Strangalina attenuata* (Linn.). *Ophistomis* is the New World counterpart of the subgenus *Strangalina*, but the two were considered as one by Aurivillius.

STRANGALINA LONGICORNE Gressitt sp. nov.

Elongate, narrow; antennæ reaching to last fifth of elytra.

Head, thorax, and abdomen black; ventral surface of thorax and abdomen clothed with silvery brown pubescence; pronotum slightly pubescent; elytra clothed with very short, suberect, brownish-black bristles. Antennæ with the first seven segments and the apical segment black, eighth to tenth segments, inclusive, pale buff, clothed with pubescence of the same color. Elytra burnt ochraceous brown, having a varnished appearance with somewhat satiny reflections; each marked with a some-

what indistinct round black spot near the external margin at a point sightly before the middle; suture, external margins, and extreme apices black. Legs ochraceous brown, lighter than elytra; middle and hind tibiæ dark reddish brown, tarsi nearly black.

Head moderately long, broad across genæ, very narrow behind eyes, punctate, except on middle of frons; eyes prominent, subglobular; frons short, clypeus large. Antennæ long and thick, reaching to last fifth of elytra, scape only slightly thickened towards apex, slightly shorter than third segment, fifth segment longer than fourth, fourth subequal to sixth and seventh, eighth and ninth subequal, tenth and eleventh subequal. Prothorax long, narrow, three-fifths as wide at apex as at base, not swollen at middle, slightly constricted between middle and external angles of base, which are acute; base four-fifths as broad as elytra at base, surface finely punctate. Scutellum narrow and long. Elytra long, very narrow towards apices; surface finely punctate; apices obliquely truncate. Ventral surface of body smooth, satiny, very minutely punctate; abdomen long, segments narrow. Legs long, hind tibiæ longer than femora; first segment of middle tarsi slightly longer than remaining segments united; first segment of hind tarsi one-third longer than remaining segments united; hind tarsi as long as hind femora; hind femora lacking about three millimeters of reaching elytra apices.

Length, 16 to 18 millimeters; breadth, 3.4 to 4.

Holotype, female, No. 50566, United States National Museum, and one paratype, also female, in author's collection, taken at Gusuku, Amami-Oshima Island, Loochoo Isands, Japan, July 10 and 11, 1932, by the author.

LAMIINÆ

Genus MONOCHAMMUS Guerin-Meneville

MONOCHAMMUS FILICORNIS Gressitt sp. nov.

Very similar to *M. bimaculatus* Gahan. Small, narrow, antennæ very long and fine. Mouse gray, mottled with pale fawn on elytra; each elytron marked with a round, satiny black spot slightly behind the middle and closer to the external margin; scutellum light brown; antennal segments basally annulated with light gray, which is lighter in the female.

Head narrow, impunctate, microgranulose on occiput, clothed with tawny gray adpressed hairs, sulcate between eyes; eyes

small, hardly reaching to middle of genæ, constricted almost into two parts behind antennal supports, fairly closely approximate dorsally; frons narrow, convex, subrectangular, nearly square; genæ not prominent; clypeus short, amber-colored; labrum rectangular, dark brown, with several erect black hairs; palpi black. Antennæ very long; in male three and one-third times as long as body, scape long, three-fifths as long as third segment, cicatrix only moderately prominent, third to tenth segments subequal in length, eleventh segment three-fourths as long as body; in female two and one-third times as long as body, third to sixth segments subequal in length, seventh to tenth successively diminishing, eleventh segment as long as second and third combined. Prothorax cylindrical, slightly shorter than broad, very slightly broader basally than apically; surface fairly smooth, three slight raised points noticeable on disk in male; lateral tubercles very short, barely perceptible. Scutellum very short. Elytra nearly straight-sided, rounded posteriorly; punctate, more noticeably on anterior portion of disk; broadest at humerus, narrowed more in male. Legs short, gray; hind tarsi three-fourths as long as hind femora; first segments of middle tarsi noticeably shorter than the two following segments combined; first segment of hind tarsi considerably shorter than terminal segment. Ventral surface of body grayish brown with a thin pale buff pubescence.

Length, 12 to 14 millimeters; breadth, 3.5 to 4.

Holotype, male, No. 50564, United States National Museum, and allotype, female, and one paratype in author's collection, all taken at Horisha, Formosa, May 25, 1932, by the author.

This species, although very similar in markings and very closely related to *Monochammus bimaculatus* Gahan, of India, is more similar in form and proportions to *M. subfasciatus* Bates, of Japan. *M. filicornis* differs from *bimaculatus* in its smaller size, narrower body, much longer and finer antennæ, in having the elytral spots rounder and placed more posteriorly, and in being gray instead of a rusty color. Furthermore, in the former the prothorax is smoother, with the lateral tubercles much shorter, and the scutellum is decidedly of smaller proportion.

Schwarzer [3] doubtfully recorded this species as *M. bimaculatus* Gahan from a single imperfect specimen, and the error has

[3] Ent. Blätter, 1925.

been perpetuated. This is the common species on the island, and *M. bimaculatus* is being absent from the fauna.

Genus MELANAUSTER Thompson

MELANAUSTER FLAVOMACULATUS Gressitt sp. nov.

Black, glabrous, marked with spots of dense, closely adpressed, pale yellow pubescence on scútellum, elytra, and posterior margin of metasternum.

Head black, minutely punctate; frons squarish, medially grooved; eyes small, narrow; mandibles short. Antennæ black, one-fourth their length longer than body; scape short, two-thirds as long as third segment, enlarged and rounded at apex, clothed with a few short bristles; remaining segments smooth and naked, nonannulated, except for third segment which is lightly pale at base; third segment slightly incurved at middle, one and one-third times as long as fourth; fourth one and one-fourth times as long as fifth; fifth to ninth subequal; tenth shorter than ninth; eleventh one and two-thirds times as long as tenth. Prothorax shiny black, short, very finely punctate except on disk, very smooth, with only a single slight swelling near the middle of the posterior margin and a very shallow longitudinal groove along the dorsal midline; armed at each side with an obtuse, blunt, cone-shaped tubercle. Scutellum rounded, broader than long; entirely clothed with yellow pubescence. Elytra short, each four times as long as broad, parallel for most of their length, rounded at apices; smooth, glossy black, very minutely punctate; each marked with about twelve large, rounded, yellow spots, covering one-third of surface and placed in five transverse bands, some of the spots coalescing; the first band is of three round spots, the second, third, and fourth bands are of two spots each, the outer ones broader and meeting the external margin, the inner ones more or less near the suture, the last band is of three spots forming a triangle, one spot near the apex, one anteriorly adjacent to the suture, and the last small and adjacent to the external margin; the first band close to the base of elytra with the innermost spot adjacent to the scutellum, the second band close to the first, a broad space between the second and third bands, and the third band slightly behind the middle. Ventral surface of body black, thinly clothed with pale scales, which are thicker on posterior margins of first and second abdominal sterna, a pale yellow band on posterior margin of metasternum. Legs short, thick; thinly clothed with gray scales, thickest on tarsi.

Length, 23.5 millimeters; breadth, 8.5.

Holotype, a unique specimen, probably a female, in the collection of M. Kato, of Tokyo, and taken by him at Karapin, Formosa, August 19, 1923.

JAPANESE NAMES OF NEW SPECIES

1. *Cerambyx minutum* sp. nov. Komiyama-kamikiri.
2. *Eustrangalis viridipennis* sp. nov. Aobane-hana-kamikiri.
3. *Strangalina gracilis* sp. nov. Oshima-hoso-hana-kamikiri.
4. *Strangalina longicorne* sp. nov. Higenaga-hoso-hana-kamikiri.
5. *Monochammus filicornis* sp. nov. Futamon-higenaga-kamikiri.
6. *Melanauster flavomaculatus* sp. nov. Kiboshi-gomadara-kamikiri.

ERRATUM

VOLUME 54

In the article by K. M. Heller, pages 279 to 307, the line at the side of each text figure, which is intended to show the actual size, should have been one-half as long as it is; in other words, the text figures are about twice natural size.

INDEX

[New names and new combinations are printed in boldface.]

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